

## 1. Measuring genome-wide transcription rates in the *Caenorhabditis elegans* embryo

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Rapid transcription is critical in cells that have to respond quickly to environmental and developmental cues. In the dividing embryo, developmental factors need to accumulate to threshold levels to specify cell fate, despite the constraints of short cell cycles. Early zygotic genes in many species have short primary transcripts, suggesting that they have evolved to sustain rapid transcription. High rates of PolII loading and elongation direct patterning in *Drosophila* embryos, indicating the importance of rapid mRNA accumulation during development. Yet, genome-wide mRNA accumulation rates have not been measured in individual embryonic cell types.

The *C. elegans* embryo is an ideal system to examine temporal transcription in single cells, as it develops with an invariant lineage and shows rapid changes in zygotic transcripts. For example, the endodermal specification factor *end-3* accumulates from 0 to ~600 mRNAs in 15 minutes, in a single cell. We hypothesize that controlled rapid accumulation is a common feature of early zygotic regulators, which drives robust cell fate specification. We performed single cell RNA sequencing (scRNA-seq) on ~15,000 embryonic cells and identified distinct transcription profiles in early and late progenitors, including many genes with rapid changes. We are now measuring transcript accumulation rates by combining metabolic labeling of RNA with scRNA-seq. The total reads from labeled and unlabeled RNA, and known expression patterns of zygotic factors will allow us to estimate the transcription rates and mRNA abundances of all genes in each embryonic cell. This strategy for identifying recently transcribed RNAs can also be used to refine worm embryonic lineage maps and to find conserved properties of transcription across developmental systems.

## **2. Diet-induced circadian enhancer remodeling synchronizes opposing hepatic lipid metabolic processes**

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Overnutrition disrupts circadian metabolic rhythms by mechanisms that are not well understood. Here we show that diet-induced obesity (DIO) causes massive remodeling of circadian enhancer activity in mouse liver, triggering synchronous high amplitude circadian rhythms of both fatty acid (FA) synthesis and oxidation. SREBP expression was rhythmically induced by DIO, leading to circadian FA synthesis and, surprisingly, FA oxidation (FAO). DIO similarly caused a high amplitude circadian rhythm of PPAR $\alpha$ , which was also required for FAO. Provision of a pharmacological activator of PPAR $\alpha$  abrogated the requirement of SREBP for FAO (but not FA synthesis), suggesting that SREBP indirectly controls FAO via production of endogenous PPAR $\alpha$  ligands. The high amplitude rhythm of PPAR $\alpha$  imparted time-of-day-dependent responsiveness to lipid-lowering drugs. Thus, acquisition of rhythmicity for non-core clock components PPAR $\alpha$  and SREBP1 remodels metabolic gene transcription in response to overnutrition and enables a chronopharmacological approach to metabolic disorders.

### **3. AAV-progranulin delivery to a mouse model of progranulin deficiency causes T cell-mediated hippocampal degeneration**

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Adeno-associated virus (AAV)-mediated gene replacement is emerging as a safe and effective means of correcting single-gene mutations, and use of AAV vectors for treatment of diseases of the CNS is increasing. AAV-mediated progranulin gene (GRN) delivery has been proposed as a treatment for GRN-deficient frontotemporal dementia (FTD) and neuronal ceroid lipofuscinosis (NCL), and two recent studies using focal intraparenchymal AAV-Grn delivery to brain have shown moderate success in histopathologic and behavioral rescue in mouse FTD models. Here, we used AAV9 to deliver GRN to the lateral ventricle to achieve widespread expression in the Grn null mouse brain. We found that despite a global increase in progranulin throughout many brain regions, overexpression of GRN resulted in dramatic and selective hippocampal toxicity and degeneration affecting both neurons and glia. Histologically, hippocampal degeneration was preceded by T cell infiltration and perivascular cuffing, suggesting an inflammatory component to the ensuing neuronal loss. GRN delivery with an ependymal-targeting AAV for selective secretion of progranulin into the cerebrospinal fluid (CSF) similarly resulted in T cell infiltration as well as ependymal hypertrophy. Interestingly, overexpression of GRN in wild-type animals also provoked T cell infiltration. These results call into question the safety of GRN overexpression in the CNS, with evidence for both a region-selective immune response and cellular proliferative response following GRN gene delivery. Our results highlight the importance of careful consideration of target gene biology and cellular response to overexpression in relevant animal models prior to progressing to the clinic.

#### **4. CAP2 loss activates MRTF/SRF signaling in cardiomyocytes**

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Dilated cardiomyopathy (DCM) is most commonly caused by mutations in actin cytoskeletal proteins. Yet treating the cytoskeleton directly is not possible because drugs that bind to actin are not well tolerated. We tested treating a cytoskeletal model of DCM by targeting the SRF pathway. CAP2 is an actin binding protein. CAP2 knockout mice (KO), either whole body or cardiomyocyte specific, develop dilated cardiomyopathy with cardiac conduction disease. RNA-seq analysis of CAP2 KO hearts revealed over-activation of SRF regulated genes such as Myl-9 and Acta-2. The activated SRF signaling is specific to cardiomyocytes and seen in mice prior to the emergence of cardiac disease. To test if we could treat CAP2 KO mice, we synthesized and tested the SRF inhibitor CCG-1423-8u. CCG-1423-8u reduced expression of the SRF targets Myl-9 and Acta-2, as well as the biomarker of heart failure, NPPA. The cardiomyocyte specific KO mice all mice died from complete heart block and or heart failure with a mean survival of 100 days, but treated KO mice maintained cardiac function and subsequently survived for 115 days. These results suggest that some forms of sudden cardiac death and cardiac conduction disease are under cytoskeletal stress and that relieving the stress by reducing signaling through SRF may benefit DCM.

## **5. Blue light stimulation of lateral pontine parabrachial neurons depresses hypoglossal nerve activity in rats**

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In obstructive sleep apnea, activity of upper airway muscles, which are innervated by the hypoglossal (XII) nerve, declines during sleep, leading to upper airway obstruction. In rats, this decline in activity of noradrenergic (NE) neurons from pontine A7, subC and A5, reduces activation of XII motoneurons. Our goal was to assess influence of optogenetic stimulation of pontine NE cell groups and its effect on XII activity. 9 Long Evans TH-Cre<sup>±</sup> rats received unilateral injections of 200-400 nl of AAV9-ChR2-eYFP into A7 and subC. After 6 weeks, under acutely anesthetized conditions, XII nerve activity and other cardio-respiratory parameters were monitored. A light fiber was stereotaxically positioned in the pons and blue light (473nm) stimulation of 10Hz, 7ms pulse width (1 min duration) and power of 45-100mW, was used. An additional 2 Cre rats did not receive the viral vector but were subjected to the same experimental paradigm. The animals were perfused and brain sections were processed for co-localization of enhanced yellow fluorescent protein (eYFP) and tyrosine hydroxylase (TH). In 6 of the 11 animals, stimulation resulted in a depression of XII nerve activity, which was preferentially elicited from the lateral parabrachial (PB) pontine region, by 18-70% of the pre-stimulation activity. In 3 of these animals, there was also a reduction of respiratory rate and an increase in blood pressure. In conclusion, blue light is an effective stimulus to assess the influence of lateral pontine region on XII nerve activity. Since the effects occurred independently of the presence of channelrhodopsin-2 (ChR2), it suggests that TH-expressing neurons may not be the main target through which light exerted its effects. These effects bear similarity to the findings reported in earlier studies with local electrical or neurochemical stimulation applied to the pontine PB region.

## 6. Ward capacity strain: a novel predictor of 30-day readmissions for intensive care unit survivors

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Intensive care unit (ICU) survivors are at risk for 30-day readmissions, but contributing organizational factors are unknown. Most survivors transfer to wards before discharge. We hypothesized that ward capacity strain – when demand for clinical resources exceeds availability – on the last hospital day predicts 30-day readmissions. A retrospective cohort study of ICU survivors in 3 hospitals 2014-2015 was done. Strain variables included patient volume (admissions, discharges, census) and staff workload (transports, medications, respiratory therapy orders, transfusions, telemetry monitoring, high acuity patients, ICUs transfers) on the last hospital day. The outcome was 30-day readmissions. We performed logistic regression using patient variables (age, gender, race, insurance, Elixhauser, length of stay prior to ward admission) and adding strain variables to predict 30-day readmissions overall and stratified by wards. We performed penalized logistic regression to identify variables most predictive of 30-day readmissions. The study population included 18,710 visits in 33 wards. Median age was 63 years (IQR 51-73), 56% were male, and 61% were white. Overall, models with patient variables and adding strain variables had similar predictions ( $c=0.61$  [95% CI 0.59-0.61] vs  $0.62$  [0.60-0.63],  $p=0.03$ ). Among individual wards, strain variables improved prediction ( $c$  increased by 0.01-0.15), with 9 of 33 wards significantly increasing. Medications, discharges, and census were 3 of the 5 strongest predictors. Although unchanged overall, strain metrics improved 30-day readmission predictions in ~1/3 wards. Future studies are needed to externally validate our findings.

## 7. The action of molecular machines revealed by HX-MS

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We used hydrogen-exchange mass spectrometry (HX MS) to study the working mechanisms of important molecular machines. The high-quality HX MS data presented here reveals detailed information on molecular machines in action that is otherwise inaccessible to other methods. We present two examples to demonstrate the versatility and power of this method.

First, we show assisted folding of maltose binding protein (MBP) by GroEL, a ring-shaped homo-tetradecamer that uses ATP binding and hydrolysis to capture and encapsulate denatured substrate proteins into an enclosed chamber for it to re-gain its native conformation. We've found GroEL rescues the folding defect of an MBP slow folding mutant (V8G) by restoring the stability of an important on-pathway folding intermediate via simple encapsulation.

Second, we show structural dynamics of a AAA+ disaggregase Hsp104 and its importance in functioning as a molecular machine. Its function in solubilizing amorphous aggregates and amyloids hinge upon cooperative effort from the two conserved AAA nucleotide binding domains (NBDs) in response to ATP binding and hydrolysis. HX MS measurements on Hsp104 performed under native conditions provide not only an independent check on the published static structure but also reveal critical function-related structural dynamics. Binding of different types of nucleotides induce different response all throughout the whole molecule and it indicates the existence of an allosteric pathway that span the entire Hsp104 molecule from the nucleotide binding pocket (Walker A domain) all the way to the 'business end', i.e. the pore loops and the hexamer interface.

## 8. ICU capacity strain and outcomes of critical illness in a resource-limited setting: A two-center study in South Africa

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**Objective:** To measure the association of Intensive Care Unit (ICU) capacity strain with processes of care and outcomes of critical illness in a resource-limited setting.

**Design:** Retrospective cohort study. Setting: Two public hospitals in South Africa. Patients: 5,332 patients referred for ICU admission and captured in the country's first published multicenter electronic critical care database.

**Measurements:** We assessed the association between multiple ICU capacity strain metrics (ICU occupancy, turnover, census acuity, and referral burden) at different exposure time points (ICU referral, admission, and/or, discharge) with clinical and process-of-care outcomes. The association of ICU capacity strain at the time of ICU admission with ICU length of stay (LOS), the primary outcome, was analyzed with a multivariable Cox proportional hazard model. Secondary outcomes of ICU triage decision (with strain at ICU referral), ICU mortality (with strain at ICU admission), and ICU LOS (with strain at ICU discharge), were analyzed with linear and logistic multivariable regression.

**Main Results:** No measure of ICU capacity strain at the time of ICU admission was associated with ICU LOS, the primary outcome. ICU occupancy at the time of ICU admission was associated with increased odds of ICU mortality (OR = 1.07, 95% CI 1.02-1.11, p = 0.004), a secondary outcome. Conclusions: In a resource-limited setting, ICU capacity strain at the time of ICU admission was not associated with ICU LOS. In secondary analyses, higher ICU occupancy at the time of ICU admission, but not other measures of capacity strain, was associated with increased odds of ICU mortality with an effect similar to prior findings in resource-rich settings.

Using a retrospective cohort study we measured the association of Intensive Care Unit (ICU) capacity strain with processes of care and outcomes of critical illness in a resource-limited setting. Using the patients from two public hospitals in South Africa. Our patient cohort consisted of 5,332 patients referred for ICU admission and captured in the country's first published multicenter electronic critical care database.

We assessed the association between multiple ICU capacity strain metrics (ICU occupancy, turnover, census acuity, and referral burden) at different exposure time points (ICU referral, admission, and/or, discharge) with clinical and process-of-care outcomes. The association of ICU capacity strain at the time of ICU admission with ICU length of stay (LOS), the primary outcome, was analyzed with a multivariable Cox proportional hazard model. Secondary outcomes of ICU triage decision (with strain at ICU referral), ICU mortality (with strain at ICU admission), and ICU LOS with strain at ICU discharge), were analyzed with linear and logistic multivariable regression.

We find that no measure of ICU capacity strain at the time of ICU admission was associated with ICU LOS, the primary outcome. ICU occupancy at the time of ICU admission was associated with increased odds of ICU mortality (OR = 1.07, 95% CI 1.02-1.11, p = 0.004), a secondary outcome. Therefore, in a resource-limited setting, ICU capacity strain at the time of ICU admission was not associated with ICU LOS. In secondary analyses, higher ICU occupancy at the time of ICU admission, but not other measures of capacity strain, was associated with increased odds of ICU mortality with an effect similar to prior findings in resource-rich settings.

## 9. Evaluating LRRK2 as a therapeutic target in Parkinson's disease

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder. The clinical diagnosis of PD is confirmed post-mortem by the presence of intracytoplasmic inclusions termed Lewy bodies, which consist primarily of the synaptic protein  $\alpha$ -synuclein. While  $\alpha$ -synuclein is thought to be pathogenic in this largely sporadic disease, mutations in several genes can increase the lifetime risk of developing disease. The most commonly mutated gene in PD is leucine-rich repeat kinase (*LRRK2*). *LRRK2* is a broadly-expressed protein with unclear function. It has kinase, GTPase and scaffolding domains and has been implicated in membrane trafficking and cytoskeletal modeling. The most common PD-linked mutation, G2019S, leads to elevated kinase activity, so *LRRK2* inhibitors have been the subjects of intense drug development for PD. However, the lack of a reliable preclinical disease model for *LRRK2* dysfunction has been a major challenge for the field. We developed cell and animal models to evaluate whether mutant *LRRK2*\*G2019S expression exacerbates the extent or spread of  $\alpha$ -synuclein pathology. We then utilized these models to test the efficacy of several top *LRRK2* inhibitors to affect  $\alpha$ -synuclein pathology. Our data represent important preclinical observations that will inform the development of *LRRK2* inhibitors for use in PD patients with and without mutations in *LRRK2*.

## 10. A novel treatment approach for targeting Cyclin E over-expressing ovarian cancers

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Cyclin E overexpressing (CCNE1<sup>HIGH</sup>) ovarian cancers are with poor survival and platinum resistance, lacking of effective therapies. Aberrant expression of Cyclin E leads to unscheduled entry into S phase, premature origin firing, nucleotide depletion, and replication fork stress. The imbalances created by this abnormal progression leads to an increased reliance on cell cycle checkpoint regulators, such as WEE1 and ATR, which counter some of the untoward effects of Cyclin E overexpression. WEE1 is a dual specificity kinase that regulates cell cycle progression by inhibiting both CDK2 and CDK1, thereby inhibiting progression from G<sub>1</sub> to S and G<sub>2</sub> to M phases, respectively. ATR kinase protects the replication fork from collapse thereby inhibiting G<sub>2</sub>/M progression so DNA can repair. This implies the promise of WEE1i and ATRi in selective killing CCNE1<sup>HIGH</sup> tumors as a rational therapeutic strategy. We tested drug effects on survival, colony formation, cell cycle and apoptosis in vitro and in patient-derived xenograft (PDX) models. Combination of WEE1i with ATRi (WEE1i-ATRi) synergistically decreases cell viability and colony formation in CCNE1<sup>HIGH</sup> HGSOC cells. WEE1i-ATRi dramatically increased cell apoptosis, decreased S phase cells and arrested cells at G<sub>2</sub>/M phase. The combination treatment increased double strand DNA break and replication stress. Also, combination inhibition of WEE1 and ATR promoted mitotic catastrophe. Finally, combination WEE1-ATRi or sequential treatments is tolerable and results in a 4-fold increase in survival compared to standard chemotherapy or monotherapy in a *CCNE1* amplified HGSOC PDX model. Our studies developed a novel combination treatment approach for targeting Cyclin E over-expressing ovarian cancers.

## 11. Novel interactions between skin commensal and pathogenic species

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The skin is host to a community of microbial species, the skin microbiome, which is hypothesized to contribute to the functionality of the skin and provide protection against pathogens. However, the individual contributions of skin microbiota that provide protection against pathogens or contribute to different disease states are widely unknown. In fact, basic functional analysis of the majority of microbial species colonizing healthy skin has not been performed.

To address this knowledge gap, we used skin 16S rRNA amplicon and metagenomic sequencing datasets to inform a targeted culture approach to generate a diverse culture collection of over 175 skin bacterial isolates. We compared isolates from the most abundant skin genera to nine *Staphylococcus aureus* clinical isolates cultured from atopic dermatitis lesions and the common soft tissue infection strain, Methicillin Resistant *Staphylococcus aureus* (MRSA). Growth dynamic analysis revealed variable patterns between commensals and pathogens. Next, we hypothesized that a network of commensal microbial interactions underlies functional colonization resistance. Therefore, we co-cultured isolates, both commensal and pathogenic, to determine which could coexist in a community. Mapping these interactions through network analysis revealed that pathogenic isolates commonly prevent the growth of commensal isolates and revealed some commensal species that prevented the growth of pathogenic isolates. To further characterize these interactions, we used a number of computational tools including metagenomic profiling, genome annotation, and gene prediction. Ultimately, this work will provide insight into the individual and group functionality of bacterial skin commensals ability to protect against pathogens.

## **12. A model system for advanced glycation end product-related structural degeneration of bioprosthetic valves**

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Bioprosthetic heart valves (BHV) are the preferred replacement due to their relatively non-thrombogenic properties, compared to mechanical valves that require anticoagulation. However, structural degeneration limits BHV lifespan, necessitating reoperation. Thus, there is significant clinical interest in BHV degeneration mechanisms. Advanced glycation end products (AGE) are a family of compounds formed by non-enzymatic, post-translational protein glycation. AGE have been correlated with soft tissue pathologies, e.g. atherosclerosis and renal failure. As BHV lack cells to replace collagen, BHV may be highly prone to AGE buildup. We hypothesized AGE promote structural degeneration by changing the BHV's mechanical properties and that blood proteins enhance this effect as carriers and binding sites. Because explanted BHV are unsuitable for mechanical testing, a model system was developed to test this hypothesis. Our model system used glutaraldehyde-fixed collagen sponges due to their homogeneity. We incubated sponge samples in phosphate buffered saline (PBS) with: glyoxal (an AGE precursor), bovine serum albumin (BSA, a serum protein), and glyoxal plus BSA. Collagenase assays evaluated collagen crosslink integrity. Uniaxial testing quantified stress relaxation and stiffness. Glyoxal incubation decreased collagenase susceptibility suggesting enhanced crosslinking beyond glutaraldehyde crosslinks. All 3 experimental groups showed significantly diminished relaxation and ~10% stiffness increase. Glyoxal and BSA independently and combined caused significant mechanical modifications in our model. By making the substrate stiffer and less capable of dissipating stress, the material is likely more prone to structural degeneration.

### 13. A tissue-engineered rostral migratory stream for directed neuronal replacement

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Regeneration after brain injury is hindered by a dearth of new neurons in the mature brain. Neurogenesis occurs throughout life in humans and other mammals, but is limited to the subventricular zone (SVZ) and dentate gyrus. Throughout adulthood, the rostral migratory stream (RMS) facilitates migration and maturation of neuroblasts from the SVZ to the olfactory bulb, where they integrate into circuitry to replace lost neurons. In some cases, injury results in migration of neuroblasts out of the RMS toward affected areas, but this response is insufficient, and factors like the glial scar can impede the process. We have developed techniques for fabricating aligned astrocytic bundles mimicking the organization of the glial tubes that constitute the RMS. These micro-tissue astrocytic constructs (MACs) are a new approach to neuroregeneration, designed as a living scaffold to redirect endogenous neuroblasts from the SVZ, through the glial scar, and into brain lesions. Current efforts are focused on characterizing the functional properties and surface cues in MAC versus native glial tube astrocytes. We are also utilizing MACs as a versatile *in vitro* platform to model RMS functionality by studying the effects of MACs on neural stem cell migration, differentiation, and maturation. With future development, these implantable constructs may facilitate neuroregeneration following brain injury *in vivo* by driving endogenous neuroblast migration and maturation from the SVZ to enable the gradual yet sustained repopulation of neuronal populations lost due to focal brain injury.

#### **14. Allogeneic mesenchymal stem cells derived extracellular vesicles as a superior immunosuppressant in murine arthritis therapy**

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Mesenchymal stem cells (MSCs) are creating promising new options for autoimmune disorders. MSCs utilize multiple mechanisms to modulate immune cells, and extracellular vesicles (EVs) have recently been recognized as important intercellular mediators, in this study we tested whether EVs derived from allogeneic MSCs would have therapeutic potential in comparison with their parent cells in murine collagen-induced arthritis model. At the time of primary immunization with type II collagen, DBA/1 mice were systemically infused with phosphate-buffered saline (PBS) (sham), EVs derived from 1(10)<sup>6</sup> allogeneic bone marrow mesenchymal stem cells (BMMSCs), and 1(10)<sup>6</sup> BMMSCs as control. Therapeutic efficacy was determined by arthritis activity, joint histology, and  $\mu$ CT imaging. Serum cytokine levels, as well as T cell phenotypes in blood, spleen and draining lymph nodes were analyzed. Naïve CD4<sup>+</sup> T cells were isolated and co-cultured with BMMSCs or EVs to assess activation and differentiation. EVs outperformed BMMSCs in three aspects: clinically, EVs treated mice had delayed onset and milder arthritis score; histologically, with decreased joint inflammation, pannus formation, collagen disruption, IL-17<sup>+</sup> cells infiltration, as well as  $\mu$ CT visual score; and immunologically, with lower serum IL-6 and IL-17, as well as decreased Th17 and activated CD4<sup>+</sup> T cells in the circulation and lymphoid organs. When co-cultured in vitro and compared with BMMSCs, EVs decreased Ca<sup>2+</sup> release upon CD3/28 stimulation and greatly suppressed naïve T cells activation, and suppressed Th17 differentiation. Collectively, these data demonstrated the outstanding therapeutic efficacy of EVs derived from allogeneic MSCs, and provided a new strategy for stem cell-based immunotherapy.

## 15. Ndfip proteins target Robo receptors for degradation and allow commissural axons to cross the midline in the developing spinal cord

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In bilaterally symmetric animals, commissural axons initially respond to attractive signals at the midline, but once they cross, they become sensitive to repulsive cues. This switch prevents axons from re-entering the midline, allowing them to turn longitudinally to reach their synaptic targets. In insects and mammals, negative regulation of the expression and activity of Roundabout (Robo) receptors prevents premature response to Slit. In *Drosophila* commissural axons, Robo1 expression and activity are regulated by the endosomal sorting receptor Commissureless (Comm), which prevents Robo1 surface expression by diverting newly synthesized Robo1 into the late endosomal compartment. In contrast to Slit ligands and Robo receptors, the *comm* gene is apparently not conserved outside of dipteran insects. This raises the critical question of how Robo1 levels and activity are negatively regulated in commissural axons prior to crossing the floor plate in the mammalian spinal cord. We have identified two mammalian Nedd-4 interacting proteins, Ndfip1 and Ndfip2, which act analogously to Comm by recruiting mammalian Robo1 to endosomes. In addition, Ndfip proteins promote Robo1 ubiquitylation and degradation through the recruitment of Nedd4 E3 Ubiquitin ligases. Ndfip proteins are expressed in commissural axons in the developing spinal cord, and removal of Ndfip1 or Ndfip2 results in an increase in the expression of Robo1 and a failure of some spinal commissural axons to cross the floor plate. Our results demonstrate the conservation of a Robo1 intracellular sorting mechanism between flies and mammals to avoid premature responsiveness to Slit.

## 16. Sympathetic nerve-control of bone homeostasis *via* mesenchymal stem cell-to-osteoclast transfer of exosomal microRNA-21

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**Background and Rationale:** The integrity of the sympathetic nervous system (SNS) is necessary to bone homeostasis, but the mechanisms are not clear. Mesenchymal stem cells (MSCs) and microRNAs have emerged as pivotal cellular and molecular regulators in mediating bone disorders (*e.g.* osteoporosis) and facilitating regeneration. We hypothesized that SNS control of bone occurs *via* microRNA-mediated MSC function.

**Methods:** A combined methodology using behavioral, surgical, pharmacological and genetic manipulations of SNS and bone was applied. Cellular and molecular approaches were performed for mechanistic experiments.

**Results:** SNS integrity through  $\beta$ 2-adrenergic (Ad $\beta$ 2) signals critically regulates bone mass in normal and depressed mice, in which both osteogenesis of MSCs and MSC-induced osteoclastogenesis contributes. Among all mouse microRNAs in bone and MSCs, miR-21 demonstrated the largest expression changes upon SNS regulation from array data. Importantly, global and conditional miR-21 deficiency blocked SNS activation-induced osteoporosis *in vivo* through normalizing MSC-mediated bone remodeling. Mechanistically, miR-21 transcription was inducible only in MSCs through Ad $\beta$ 2 activation of C-fos binding to its promoter region, and functioned as an osteogenic stimulator. However, the SNS signal enhanced miR-21 transfer from MSCs to osteoclasts *via* exosomes, thus promoting osteoclastogenesis by targeting programmed cell death 4 (Pdc4), while at the same time inhibiting osteogenesis by targeting Sprouty 1 (Spry1).

**Conclusions:** We discovered that the SNS regulates bone *via* MSC-transfer of miR-21 to influence both arms of bone remodeling through exosomes. These findings reveal the nerve-control of organ homeostasis by stem cell-based communication, paving an avenue for therapeutic applications.

## 17. Multiple molecular mechanisms rescue mtDNA disease in *C. elegans*

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Mitochondria carry their own genome (mtDNA) to facilitate energy production. Pathogenic mutations in mtDNA can accumulate during aging or be inherited by children. These mutations can disrupt energy production and lead to diseases that impact high energy-consuming cells like neurons and muscle fibers. Currently, there are no cures for mtDNA disease. The two major impediments in developing new treatments are (i) the lack of animal models and (ii) the lack of cost-effective ways to identify new therapeutics.

To address these concerns, we developed several error-prone alleles of *polg-1*, the polymerase that replicates mtDNA. Using these mutator worms, we also generated several stable mtDNA mutant strains. This is of marked importance because specific mtDNA mutations are known to cause disease in humans but creating animal models have been very tricky since there are 10s to 100s of mtDNA molecules in a cell. We tested the *polg-1* mutant strains and found that the *polg-1* mutants exhibit hallmark features of mtDNA disease in humans, which include mtDNA instability, mitochondrial dysfunction, and a loss of neuromuscular function. Therefore, we used the mutator worms in a small, targeted RNAi screen of 130 genes and found 22 candidates that rescued mtDNA disease. Results of the screen and studies with genetic mutants further showed that reducing the IGF/Insulin pathway, reducing mitophagy, and constitutively activating the mitochondrial unfolded protein response improved the disease pathology in the *polg-1* mutants. In our study, we were able to generate new mtDNA disease models and identify new targets for therapy. Currently, we are studying the role of reducing insulin signaling in the *POLG<sup>D257A</sup>* mouse model.

## **18. Mitochondrial deficits in human iPSC-derived neurons from patients with 22q11.2 deletion syndrome and schizophrenia**

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Schizophrenia (SZ) is a highly heterogeneous disorder in both its symptoms and its causes. The strongest of the genetic risk factors for SZ is the hemizygous microdeletion at chromosome 22q11.2 (22q11DS) that confers a nearly 25-fold increased risk. Interestingly, 6 of the roughly 40 genes directly disrupted in 22q11DS encode for mitochondrial localizing proteins. Here, we studied four Induced Pluripotent Stem Cell (iPSC) lines from patients with 22q11DS and SZ, and five lines from age and sex-matched healthy individuals. In forebrain excitatory neuron-like cells derived from these lines, we found that the patient-derived neurons showed significantly decreased complex I and IV enzyme activity and decreased ATP levels. As expected, MRPL40 protein levels were reduced. Remarkably, we found that the protein products of several mitochondrial DNA-encoded genes were significantly reduced in patient iPSC-derived neurons, while no difference was observed in the mRNA level of related mitochondrial DNA-encoded genes. Additionally, these results were also present in MRPL40<sup>+/-</sup> iPSC derived neurons. Our data indicate that MRPL40 haploinsufficiency contributes to deficits of mitochondrial-encoded gene translation, which leads to reduction of complex I and IV enzyme activity and decreased ATP levels in iPSC-derived neurons.

## **19. Pembrolizumab enhances efficacy of autologous TIL therapy against ovarian cancer**

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Ovarian cancer is the 5<sup>th</sup> leading cause of cancer related deaths among women, with the vast majority diagnosed as high-grade serous ovarian cancer (HGSOC). The standard front-line therapy for HGSOC is tumor-debulking surgery combined with platinum-based chemotherapy. Recurrence develops in nearly 90% of cases, often due to chemotherapy resistance; thus, new therapeutic strategies are necessary for ovarian cancer patients. The presence of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment is associated with improved progression free survival. However, the advantage of the immune checkpoint blockade remains unclear. The goal of this study was to determine if addition of the checkpoint inhibitor Pembrolizumab increased efficacy of autologous TIL transfer in patient-matched orthotopic patient-derived xenograph (PDX) models of ovarian cancer. TILs were isolated and expanded from two individual patient tumors, one of which was platinum resistant. Evaluation of TILs co-cultured with autologous tumor cells revealed HLA-specific increased IFN- $\gamma$  production and increased expression of the T cell activation marker CD69. Moreover, TILs in combination with Pembrolizumab significantly increased patient-matched tumor lysis. These results indicate that TILs are reactive and tumoricidal against autologous tumor cells. Administration of reactive autologous TILs in patient-matched PDX models resulted in increased survival, with greater survival in groups that received autologous TILs in combination with Pembrolizumab. Overall, this study demonstrates that adoptive TIL therapy in combination with an immune checkpoint blockade enhances survival in ovarian cancer, which could be a promising treatment option for patients with resistance to platinum chemotherapy.

## **20. Application for nanofluidic devices towards single-cell proteomics: A study with *Xenopus laevis* embryos**

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Nanofluidic technologies have enabled mass spectrometric analysis of single cells and the study of inter-cellular heterogeneity. However, most of these technologies have been applied to metabolomics and lipidomic studies because lipids and small molecules are highly ionizable and predominate the mass spectra. Here, we have combined a micropipette (pulled glass capillary) based sample collection strategy with offline sample preparation and LC-MS/MS to analyze proteins through a bottom-up proteomic strategy. Micropipettes (nanofluidic sample collection devices) are fabricated through a commercially available pipette puller. The position of the micropipette is controlled with a motorized micromanipulator and individual cells in an embryo are targeted by observation under a stereoscope. After puncturing the membrane of the target cell, the sampling is controlled pneumatically via a microinjector. Post-sampling the samples are ejected into a small tube and prepared for LC-MS/MS. This strategy has enabled analysis of >1200 proteins per *Xenopus laevis* embryonic cell. Large cell sizes enabled easy manipulation and analysis of >700 proteins from <40 nL of sample harvested from a single blastomere during stage 7 (128 cell stage) of development. This tool will enable the study of cellular differentiation during development and relate it to a fate map of a developed organism. Preliminary studies have shown that at developmental stage 2, more than 60 proteins are differentially expressed in dorsal *versus* ventral cells. The number of differentially expressed proteins increased to >80 at stage 3 of development. Investigations are currently underway to look at intercellular heterogeneity among cells at later stages of development.

## **21. T-cell priming is enhanced by maturation-dependent stiffening of the dendritic cell cortex**

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T-cell activation by dendritic cells (DCs) depends on pushing and pulling forces exerted by the T-cell actin cytoskeleton. This process is enhanced by a series of changes in the DC, collectively termed maturation. Using atomic force microscopy, we show that during maturation, DC cortical stiffness is increased via a process that depends on actin polymerization. By manipulating the stiffness of T-cell substrates using stimulatory hydrogels or DCs expressing mutant cytoskeletal proteins, we show that increasing stiffness over defined range for DC maturation lowers the agonist dose needed to initiate T-cell activation. Thus, mechanical cues function as co-stimulatory signals. Stiffness sensitivity is conserved in CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, in both naïve and effector populations. Strikingly, blood-derived T-cells lack stiffness-sensitivity, suggesting that mechanosensing can be switched off. Taken together, our data reveal that maturation-associated changes in the DC cytoskeleton alter its biophysical properties, creating a platform for enhanced mechanotransduction in interacting T-cells.

## **22. Establishing the larval zebrafish pectoral fin as a model for targeted axon regeneration**

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The vertebrate peripheral nervous system has significant capacity for axon regeneration. While work in many systems has addressed neuron-intrinsic factors that promote axon growth, little is known about how the regenerating growth cone interacts with cues in the environment to reinnervate target tissues with specificity. Targeting regrowing axons to correct tissues is critical for functional recovery after nerve injury; indeed, incorrect muscle fiber reinnervation results in inappropriate movements. The larval zebrafish pectoral fin is an ideal model system to identify novel environmental cues required for target reinnervation due to its complex anatomy. The pectoral fin is innervated by four motor nerves containing dozens of axons that branch to stereotypically-innervate specific regions of the fin in two distinct muscle layers. Using a laser to transect the nerves that innervate the pectoral fin, we can monitor regeneration in real time. We observed robust and specific regeneration of pectoral fin axons back to their original domains within two days indicating that there must be regional growth and guidance cues within the fin to guide axon growth. Easy removal of the pectoral fin allows for unbiased identification of local, injury-dependent cues in vivo in a vertebrate that are not feasible in other model systems. Here, we discuss an RNAseq approach to identify factors in the regenerating pectoral fin with expression changes after axon injury that may be required for axon growth and guidance. Using this approach as a gateway to understand the underlying molecular-genetic mechanisms that promote sustained and directed growth of regenerating axons will generate a strong foundation for therapeutic applications aimed to promote functional PNS recovery.

### **23. Maternal and early-life diet dictates bodyweight gain and alters hindbrain plasticity in offspring.**

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While previous studies highlight the detrimental impact of *in utero* and postnatal exposure to a high-fat diet (HFD) on the propensity of offspring to develop obesity, the neurobiological mechanisms underlying this outcome are not well understood. Here, we show that maternal and postnatal consumption of HFD dramatically alters the DVC cytoarchitecture via increased astrogliosis, putatively contributing to predisposition of offspring to have reduced sensitivity to satiation signals and increase bodyweight gain. Further, as recent therapeutic strategies for treating obesity in adults have focused on targeting the glucagon-like peptide-1 (GLP-1) system, here we also examine whether the GLP-1 receptor agonist liraglutide could be used in juvenile rats as an anti-obesity pharmaceutical to prevent not only adolescent but also adult obesity caused by maternal and early-life HFD overnutrition. Male and female rats were maintained on chow or HFD, according to the maternal diet. Animals received a daily subcutaneous injection of liraglutide (50µg/kg, from postnatal day [PND]30-PND40; 200µg/kg from PND40-PND60) or vehicle. Our results show that chronic administration of liraglutide in juvenile rats prevented bodyweight gain and retained a normo-glycemic profile in males but not females. These preclinical data suggest that maternal and early-life HFD increases astrogliosis in the DVC, caloric intake and bodyweight gain – a collective set of unwanted metabolic effects that appear to be treatable in male juveniles with GLP-1R agonist pharmaceutical intervention.

## **24. MondoA signaling links skeletal muscle lipid accumulation and insulin resistance**

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It is well known that insulin resistance in the obese state is associated with accumulation of a neutral lipid in non-adipose tissues. This phenomenon is particularly well-documented in skeletal muscle. The mechanistic underpinnings of this linkage are not fully understood. We hypothesized that fundamental homeostatic mechanisms exist to coordinately control cellular lipid stores and insulin-mediated glucose uptake. Previously, we found that MondoA inhibits muscle insulin signaling via activation of TXNIP and increases neutral lipid stores through an unknown mechanism. Current studies demonstrated that nutrient loading with glucose activated MondoA by triggering its localization to the nucleus. Expression of TXNIP was regulated in accordance with nuclear levels of MondoA. These results indicated that MondoA serves to deactivate insulin signaling and reduce glucose uptake to prevent cellular nutrient overload. Whole genome RNA-seq and CHIP-seq studies demonstrated that MondoA regulates target genes involved in nutrient storage pathways and insulin signaling. In addition, MondoA regulates Klf10 and Klf11, known regulators of glucose homeostasis. Muscle-specific genetic deletion of MondoA in mice was shown to improve glucose tolerance in the context of diet-induced obesity. We conclude that MondoA plays a key role as a cellular transcriptional checkpoint to control nutrient catabolism by suppressing insulin signaling and shuttling fatty acids into triglyceride stores during conditions of “plenty.” In the context of chronic activation of MondoA (caloric excess), a vicious cycle of myocyte lipid accumulation and insulin resistance ensues. Accordingly, MondoA signaling is a candidate target for strategies aimed at reducing insulin resistance and cellular lipotoxicity.

## 25. Skewed CD4 and CD8 T cell differentiation in pancreatic cancer patients

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To understand potential immune system alterations in newly diagnosed, untreated, pancreatic cancer patients and provide a foundation for immunotherapy, we profiled PBMC from pancreatic ductal adenocarcinoma (PDA) patients and age-matched healthy controls using high dimensional CyTOF analysis. We developed two immune profiling panels: a broad profiling panel that includes 45 phenotypic markers that together permit the identification and enumeration of the main innate and adaptive immune cell subsets in humans, and a deep profiling panel that includes 45 features focusing on T cell phenotype and biology. We report a 2-fold increase in monocytes, more regulatory T cells, and more plasmacytes in circulation in pancreatic cancer patients as compared to age-matched controls, as well as a bias towards cytokine-producing NK cells. Using high dimensional approaches, we observed skewed T cell differentiation in pancreatic cancer patients, with peripheral blood CD8 T cells being biased towards more CD45RA-positive CD27-positive CCR7-positive CD95-positive CD49d-positive stem cell memory cells ( $p=7 \times 10^{-5}$ ), more CD45RA-negative CD27-positive CCR7-negative effector memory cells ( $p=0.002$ ) and less CD45RA-positive CD27-negative CCR7-negative late effector memory cells ( $p=0.003$ ) as compared to age-matched controls. Strikingly, when examining T cell differentiation in human spleens, we found increased proportions of late effector memory T cells in the splenic CD8 T cell compartment of pancreatic cancer patients as compared to age-matched controls. These results suggest an impaired T cell trafficking in pancreatic cancer patients, with late memory T cells being retained in the spleen. We are now investigating the mechanisms underlying these observations, as well as their impact on T cell immunity of the cancer patients. Our goal is to understand the nature of the skewing and how any changes in the baseline immune health of the T cell compartment relate to disease progression and/or response to therapy. These studies should provide a foundation for improving therapy in pancreatic cancer patients.

## 26. Developing therapeutic protein disaggregases for neurodegenerative disease

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The accumulation of abnormal protein aggregates in the human brain is connected with neurodegenerative diseases. The mammalian protein-disaggregase system is comprised of Hsp110, Hsp70, and Hsp40 and can protect cells from proteinopathies. However, this disaggregase system has limitations and its activity declines with age. As a therapeutic strategy for the neurodegenerative disease we propose to introduce an exogenous, synthetic disaggregase machinery, based on Hsp104 that converts amyloid to soluble, functional protein. Hsp104 is a hexameric AAA+ ATPase that is critical for stress tolerance in yeast by facilitating the resolubilization of stress-damaged, aggregated proteins. The homologues of Hsp104 are highly conserved in bacteria, fungi, and plants, but absent from metazoa. The disaggregase activity of Hsp104 can be enhanced by the presence of Hsp70 and Hsp40. Hsp104 can dissolve  $\alpha$ -synuclein,  $\beta$ -amyloid, and tau aggregates, but impractically high Hsp104 concentrations are needed. We aim to rationally design potentiated Hsp104 variants that can reverse protein misfolding at low concentrations. By investigating the mechanism of Hsp104 disaggregation and its structural basis, we will engineer and evolve potentiated Hsp104 variants that eradicate  $\alpha$ -synuclein,  $\beta$ -amyloid, or tau misfolding and toxicity. We have exploited new cryo-EM structures of Hsp104 to engineer key interfaces to yield several novel Hsp104 variants with tunable activity, which can reverse protein aggregation in the presence or absence of human Hsp70 and Hsp40. These novel Hsp104 variants effectively rescue the aggregation and toxicity of diverse human neurodegenerative disease proteins and could have therapeutic utility. Moreover, our engineered Hsp104 variants provide key mechanistic insights into disaggregase function.

## **27. The nuclear receptor ERR $\gamma$ is a master regulator of cardiac developmental maturation**

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The heart undergoes dramatic developmental maturation following birth and during the postnatal period. This transition involves robust mitochondrial biogenesis coincident with changes in contractile protein isoforms and ion channel expression. This coordinated energetic and structural developmental maturation is necessary for the persistent high performance of the postnatal heart. We profiled expression of transcription factors known to regulate mitochondrial function during differentiation of human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs) and found that estrogen-related receptor gamma (ERR $\gamma$ ) was markedly induced in parallel with known cardiogenic factors. CRISPR-based gene deletion studies demonstrated that ERR $\gamma$  is necessary for mitochondrial maturation in hiPSC-CMs. Interestingly, forced expression of ERR $\gamma$  pushed the hiPSC-CMs to an adult phenotype coordinately regulating mitochondrial metabolic maturation and expression of adult structural genes. Cardiac-specific deletion of ERR $\gamma$  and ERR $\alpha$  in mice resulted in an arrest in the fetal to adult transition and 100% postnatal mortality. Disruption of ERR $\gamma/\alpha$  during the postnatal period in mouse heart demonstrated that this circuitry is necessary for full postnatal-adult transition. Whole genome RNA-sequencing and ChIP-sequencing studies indicated that ERR signaling directly coordinates adult energy metabolic and structural programs by remodeling enhancer chromatin. Moreover, ERR $\gamma$  suppresses early stage fetal and non-cardiac lineage gene programs. We conclude that ERR signaling serves as a master upstream trigger for the transition from fetal to adult programs in the developing heart. Activation of ERR signaling may prove useful as a strategy to drive hiPSC-CM in culture to full adult differentiation.

## **28. Exponential fluorescent amplification of individual nucleic acids using clampFISH probes**

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Non-enzymatic, high-gain signal amplification methods with single-cell, single-molecule resolution are in great need. We present click-amplifying FISH (clampFISH) for the fluorescent detection of nucleic acids that combines the specificity of oligonucleotides with bioorthogonal click chemistry in order to achieve high specificity and extremely high-gain (>400x) signal amplification. We show that clampFISH signal enables detection of RNA species with low magnification microscopy and separation of cells by RNA levels via flow cytometry. Additionally, we show that the modular design of clampFISH probes enables multiplexing of RNA and DNA that the locking mechanism prevents probe detachment in expansion microscopy, and that clampFISH can be applied to tissue samples.

## **29. Diagnosis of triple negative breast cancer using machine learning methods of quantitative ultrasound features**

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Triple negative (TN) breast cancers are known for aggressive biological characteristics and poor clinical outcomes. The aim of this study was to apply computer methods with ultrasound imaging to differentiate TN and non-triple negative (NTN) subtypes. 140 surgically confirmed breast cancers were classified into TN and NTN subtypes based on the expression of ER, PR, HER-2. Nine quantitative grayscale features describing margin and shape characteristics of the lesion, and three tumor vascularity features describing the magnitude of vascularity were extracted from a manually drawn region of interest on grayscale and color Doppler images. The features that showed difference ( $P < 0.05$ ) were used with logistic regression and leave-one-out cross validation to train and test the differentiation of TN and NTN masses. Diagnostic performance was measured by the area under ROC (AUC) and sensitivity and specificity measured at the Youdens index. Of the twelve grayscale and Doppler features, eight showed statistical difference ( $P < 0.002$ ) for the TN and NTN. AUC of the statistically significant GS and Doppler features when used alone was 0.850 and 0.657, respectively. The AUC increased to 0.882 when all the significant GS and CD features were used. The improvement by inclusion of Doppler features was significant ( $P < 0.0001$ ). Sensitivity and specificity of combined grayscale and Doppler was 78.26% and 85.47%, respectively. Consideration of patient age in the analysis did not improve discrimination of TN and NTN. The analysis of breast ultrasound by machine learning can achieve high level of differentiation between the TN and NTN subtypes that is comparable to the diagnostic performance by standard visual assessments of the images.

### **30. Characterization of the PAX8 transcriptional complex to identify novel therapeutic vulnerabilities for ovarian cancer**

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A central problem in the treatment of ovarian cancer remains the heterogeneity among ovarian tumors. Despite efforts to elucidate common signaling pathways among various ovarian cancer subtypes, few have led to meaningful patient stratification or to truly individualized therapies. Our proposed set of experiments is a radical departure from the conventional approach to treating ovarian cancer. PAX8, a transcription factor which identifies nearly all high grade serous ovarian cancer (HGSOC), is also the master regulator of fallopian tube development. Building off observations pointing to the fallopian tube epithelium as a major site of origin for HGSOC, we propose targeting the fallopian tube developmental program as the basis for new therapies. This possibility is supported by the observation that knockdown of PAX8 leads to apoptosis in ovarian cancer cells. We hypothesize that blocking the ability of PAX8 to influence its gene targets, either by interrupting PAX8 protein-protein interactions or by inhibiting the products of PAX8-driven signaling, has the potential to eliminate the primary growth stimulus for HGSOC. In order to achieve that goal, we have identified PAX8-interacting partners using chromatography and mass spectrometry analyses. Interactions have been validated by WB, IF, and PLA. Our results suggest that PAX8 interacts with other transcription factors and components of chromatin-remodeling complexes. Further studies will be done in order to decipher the functional contribution of each candidate PAX8-interacting protein. Inhibition of certain protein-protein interactions may provide novel avenues to abrogate PAX8 function in ovarian cancer.

### **31. Unsuspected osteochondroma-like outgrowths in the cranial base of Hereditary Multiple Exostoses patients and treatment with a BMP antagonist in mice**

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Hereditary Multiple Exostoses (HME) is a rare autosomal-dominant pediatric disorder that affects about 1 in 50,000 individuals worldwide. HME is characterized by formation of cartilaginous outgrowths—called osteochondromas—next to the growth plates of many axial and appendicular skeletal elements. Because of their size, large number and location, the osteochondromas can cause severe problems such as skeletal deformities, chronic pain and early onset osteoarthritis. Surprisingly, it is not known whether such tumors also form in endochondral elements of the craniofacial skeleton. Here, we carried out a retrospective analysis of cervical spine MRI and CT scans from 50 consecutive HME patients that included cranial skeletal images. Interestingly, nearly half of the patients displayed moderate defects or osteochondroma-like outgrowths in the cranial base and specifically in the clivus. In good correlation, osteochondromas developed in the cranial base of mutant *Ext1<sup>fl/fl</sup>;Col2-CreER* or *Ext1<sup>fl/fl</sup>;Aggrecan-CreER* mouse models of HME along the synchondrosis growth plates. Because chondrogenesis requires bone morphogenetic protein (BMP) signaling, we asked whether osteochondroma formation could be blocked by a BMP signaling antagonist. Systemic administration with LDN-193189 effectively inhibited osteochondroma growth in conditional *Ext1*-mutant mice. In vitro studies with mouse embryo chondrogenic cells clarified the mechanisms of LDN-193189 action that turned out to include decreases in canonical BMP signaling pSMAD1/5/8 effectors but interestingly, concurrent increases in such anti-chondrogenic mechanisms such as pERK1/2 and *Chordin*, *Fgf9* and *Fgf18* expression. Our study is the first to reveal that the cranial base can be affected in patients with HME and that osteochondroma formation is amenable to therapeutic drug intervention.

## 32. TSPO regulates the pathogenesis of Alzheimer's disease

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Increasing evidences have pushed inflammation and immune dysfunction to the center of the pathogenesis of AD. Clinical studies demonstrate that Translocator Protein (18kDa) (TSPO)/Peripheral Benzodiazepine Receptor (PBR), a mitochondrial transmembrane protein, plays crucial roles in neuroinflammation and neurodegenerative disease, such as AD and Parkinson's disease. However, the roles of TSPO in inflammatory response and the pathogenesis of neurodegeneration remain elusive. Our previous work using TSPO global knockout (KO) mice demonstrated that TSPO was dispensable for mouse development and immune homeostasis. Here, we investigated the roles of TSPO in neuroinflammation and A $\beta$  pathology in AD-like mouse model. Our results showed that TSPO were upregulated in brain tissues from AD/dementia patients and AD mouse model. APP/PS1 mice lacking TSPO suffered a higher A $\beta$  1-40 and A $\beta$  1-42 peptides production, and more  $\beta$ -amyloid plaques in cortex and hippocampus regions compared to APP/PS1 mice. Meanwhile, the levels of microglia and astrocyte activation were also elevated in APP/PS1 mice lacking TSPO compared to APP/PS1 mice, especially surrounding the plaques. We also found that TSPO deletion promoted inflammasome activation, IL-1 $\beta$  conversion and associated pyroptosis in peritoneal macrophages, a peripheral homogeneous cell population of microglia. Taken together, these results indicate that TSPO plays a potential protection function against the neuroinflammation and  $\beta$ -amyloid pathology. To our knowledge, this is the first phenotype identified in global TSPO KO mice.

### **33. Assessment of flow-mediated dilation by continuous cross-sectional ultrasound imaging of the brachial artery**

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Impairment of flow-mediated dilation of the brachial artery (FMD) is a marker of endothelial dysfunction. In this study we propose a novel feed-forward active contour (FFAC) algorithm with continuous monitoring of cross-sectional area (CSA) to improve reproducibility and sensitivity of FMD. BA-FMD was performed in 16 volunteers in cross-sectional and longitudinal views. Clips were recorded pre-cuff inflation and post-cuff deflation. Time-dilation curves of lumen CSA were measured using automated segmentation of BA by FFAC. %FMD was determined by ratio of peak dilation to baseline value. To evaluate reproducibility each measurement was repeated twice in two sessions one hour apart. Subject-specific and session-specific coefficient of variation for FFAC were measured and compared with longitudinal FMD measurements. CSA-FMD values were two times greater than those measured by longitudinal diameter. Session-specific FMD for FFAC at peak dilation were highly reproducible. FMDs for the two sessions were identical, 33.2% vs. 33.0% ( $p>0.05$ ) with coefficient of variation (CV) 0.4%. FMDs by conventional approach for the two sessions were 8.3% vs. 9.1% ( $p>0.05$ ) with higher CV of 7%. CV of subject-specific measurement for CSA by FFAC was  $10\% \pm 6\%$  versus  $50\% \pm 29\%$  for the conventional approach. The improvement in CV for FFAC over conventional approach was significant ( $p<0.01$ ). Correlation  $R^2$  as a metric of evaluation also showed better performance for cross-sectional imaging using FFAC.  $R^2$  for CSA by FFAC was 0.84 versus 0.21 for the conventional approach. The FMD measured by cross-sectional imaging using FFAC provides more reproducible and sensitive measurement than conventional longitudinal method.

### **34. An ensemble learning system for detecting tweets mentioning drug names**

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Due to the large volume of easily-accessible data, Twitter posts are increasingly regarded as an important source of health information that can provide unique insights into public health. A fundamental step when incorporating Twitter data for drug-related research is to automatically recognize names of drugs in tweets. Lexical matching based approaches are typically used to detect drug names, but such approaches have two primary limitations: drug names are often misspelled (*e.g.*, gemsar for gemzar) and they can be highly ambiguous (*e.g.*, Lyrica is an antiepileptic drug or a singer). We present here a drug name recognizer (DNR) based on Ensemble Learning and Deep Neural Network, which is capable of disambiguating drug names based on their context. Since no large corpora of tweets labeled with drug mentions were available, we created a biased corpus of ~15,000 tweets with a balanced number of tweets mentioning, or not mentioning, drugs names in order to make the training of our DNR possible. Since the statistical bias introduced during the creation of this corpus prevented us to use it for evaluating our DNR, we instead assessed its performance on a real-case corpus collected during an epidemiological study on pregnant women. This second corpus was made of all tweets posted by 113 Twitter users and representing ~100,000 annotated tweets with only 258 tweets mentioning drugs. On the biased corpus, our DNR demonstrated performances close to human annotators with a .91 F1 score. On the real-case corpus it obtained a .74 F1-score, a score comparable to the scores of the best systems competing in well-established named entity recognition challenges.

### **35. Multi-feature quantitative analysis of B-Mode ultrasound to characterize hepatic fibrosis**

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Staging hepatic fibrosis via biopsy is prone to reader variability and sampling error. This study quantitatively characterizes the progression of fibrosis with multi-feature, computerized analysis of B-mode ultrasound (US) in a rat model of hepatic fibrosis. Fibrosis was induced in 24 rats by ingestion of diethylnitrosamine (DEN). A control group (n=4) received no DEN. Rats were imaged at 0w, 10w and 13w. Images were analyzed quantitatively for echointensity, heterogeneity (variance within region), anisotropy (variance between regions), and hepatorenal index (HRI). Liver sections harvested and correlated with 13w US data. DEN rats' US features changed significantly from baseline by 10w ( $p < 0.001$  for all parameters), while controls remained unchanged. For echointensity, DEN baseline was equal to control (37.1,  $p = 0.54$ ) and rose to plateau at 53.3 at 10-13w. Controls remained 34.6. Similarly, HRI in DEN rats rose from 0.28 to 0.45 at 10w, and 0.51 at 13w. Control HRI remained 0.24. A different pattern emerged for variance. For anisotropy, DEN rats began at 11.8, peaked at 17.2 at 10w, then fell to 16.5 at 13w. Controls stayed 9.0. For heterogeneity, DEN rose from 194, peaked at 350 (10w), and fell to 329 (13w). Controls remained 226. 10w and 13w data were statistically equal for all features. Histology of controls showed F0, no fibrosis. DEN ranged F2-F4, significant fibrosis to cirrhosis. Spearman correlations of 13w data to METAVIR stage showed the strongest correlation with HRI ( $\rho = 0.801$ ). Computerized sonographic features tracked the progression of fibrosis and correlated with histologic stage. Future advancement of multi-feature characterization may improve classification of fibrosis clinically.

### **36. Prolonged release of Ibuprofen from a nanofibrous delivery system under physiological conditions**

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Using non-steroidal anti-inflammatory drugs (NSAIDs) to mitigate inflammation may represent a promising approach to modulate the tendon healing environment. In particular, biodegradable nanofibrous delivery systems offer an optimized environment for cellular activity while releasing soluble factors to promote tissue regeneration. Previous work confirmed the sustained release of Ibuprofen (IBP) from Labrafil-modified poly (lactic-co-glycolic) acid (PLGA) microspheres *in vitro* in phosphate buffer saline (PBS) and characterized a bilayer delivery system (BiLDS) incorporating these microspheres for localized delivery of therapeutics, but the behavior of the BiLDS in physiological conditions is unknown. Therefore, the objective of this study was to evaluate the release of IBP from both PLGA microspheres alone and within BiLDS in serum, and to elucidate their effect on primary tenocytes. We hypothesized that IBP would release at a faster rate from the microspheres and the BiLDS in serum than in PBS and that the BiLDS would not have an adverse effect on cellular viability or morphology *in vitro*. Ibuprofen was released over 14 days in a sustained manner from microspheres enclosed in the bilayer electrospun poly ( $\epsilon$ -capalactone) (PCL) scaffold design in serum, confirming the prolonged behavior of the microspheres in physiologically relevant conditions. The BiLDS components and release of IBP did not have any detrimental effects on cellular viability or morphology *in vitro*. This study identifies the therapeutic potential of a biocompatible nanofibrous delivery system for prolonged and continued released of Ibuprofen to mitigate inflammation during tendon healing. Ongoing studies are investigating the regenerative effects of the BiLDS in a rat rotator cuff injury and repair model.

### **37. Associative learning alters neurotransmitter release from the primary olfactory sensory neurons**

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Associative learning, the process that the brain assigns predictive values to sensory stimuli, is essential for the survival of animals in a dynamic environment. In addition to neuroplasticity in the cortical and limbic regions, the potential contribution from altered sensory inputs has recently been recognized, but the underlying neural mechanism remains elusive. The mammalian olfactory system is an excellent model for such investigation as primary olfactory sensory neurons (OSN) project their axons directly to the olfactory bulb (OB) glomeruli making their synaptic release subject to cortical influence and neuromodulation. In this study, pairing optical stimulation of a single M72-ChR2 (channelrhodopsin-2)-EYFP glomerulus with foot shock during the conditioning session led the mice to freeze to the optical stimulation alone during the retrieval session. We then compared OSN release probabilities by recording light evoked synaptic events in genetically-labeled, glutamatergic external tufted cells (Vglu1-tdTomato+) innervating the M72 glomeruli. We observed a significantly higher release probability of OSNs projecting to the M72 glomeruli that were fear conditioned (pair pulse ratio in percentage:  $28.01 \pm 6.96$ ,  $n=8$ ) than the control ones (unstimulated:  $54.44 \pm 4.28$ ,  $n=21$  or stimulated but unpaired with foot shock:  $55.22 \pm 4.89$ ,  $n=7$ ; one-way ANOVA,  $p < 0.01$ ), along with a significant increase in the spontaneous excitatory postsynaptic events, suggesting a change in the presynaptic release from fear-conditioned M72 OSNs. Furthermore, a positive correlation between the release probability and freezing behavior was evident in these mice. These results suggest that associative learning alters the peripheral olfactory inputs, which may contribute to the desired behavior.

### **38. Biosensor-based real-time study of kinetic and thermodynamic aspects of opioids interaction with mu-opioid receptor**

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Advances in developing opioids with minimal side effects are hampered by the poor understanding of the biophysical properties of opioid-receptor interactions, which is due to lack of methodologies for real-time quantification. Here, we engineered a water-soluble analogue of mu opioid receptor (ws-MOR) to demonstrate a label free biosensor-based platform for combined kinetic and thermodynamic characterization of opioids in real-time with high efficiency. The ws-MOR was expressed in high yield in bacteria and retained stable structural and functional features of the native MOR, including opioid binding capability. By covalently attaching the receptor to the sensor surface for surface plasmon resonance, both opioid binding affinity and the rate constants were determined. Through this novel approach, we discovered that while there is no correlation between affinities and clinical efficacies (human plasma EC<sub>50</sub>), there is strong correlation between kinetic rate constants and plasma EC<sub>50</sub> values, indicating the association and dissociation constants of opioids provide more valuable information about their clinical efficacies than equilibrium constants. Biosensor-based approach using ws-MOR also allowed collecting important thermodynamic data on opioid-MOR interactions. Morphine-MOR binding was exothermic and was essentially enthalpy driven. Our studies demonstrate previously unknown activation parameters for transition state formation of morphine binding to MOR. The method described here provides a unique approach to substantially improve the analysis of and provides detailed biophysical insights to opioid-receptor interaction.

### **39. The failing heart oxidizes ketone bodies as an energy metabolic stress defense**

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The failing heart has markedly reduced capacity for utilizing fatty acids, the primary fuel of the adult mammalian heart. This fuel metabolic disturbance leads to an energy deficit that contributes to the development of heart failure (HF). Recently, we discovered that the failing heart re-programs to utilize ketone bodies made in the liver. The role of this fuel shift as adaptive or maladaptive is unknown. To address this question, we generated mice with cardiac-specific knockout of 3-hydroxybutyrate dehydrogenase 1 (csBDH1<sup>-/-</sup>), which catalyzes the first step in the oxidation of the ketone body 3-hydroxybutyrate (3-OHB). Combined ventricular pressure overload and myocardial infarction (TAC/MI) studies demonstrated that the csBDH1<sup>-/-</sup> mice exhibit markedly worsened HF compared to wild-type controls. Wild-type mice fed a ketogenic diet exhibited a modest reduction in pathologic remodeling when subjected to TAC/MI. We next employed a well-defined canine pacing model of progressive HF to test the effects of 3-OHB supplementation. Infusion of 3-OHB during the pacing protocol increased cardiac ketone extraction and markedly reduced pathologic cardiac remodeling compared to pacing without 3-OHB infusion. The 3-OHB infusion prevented the pacing-induced reduction in cardiac output and elevation of left ventricular end-diastolic pressure and also reduced peripheral vascular resistance. Oxidation of labeled substrates indicated that the increased ketone utilization normalized the abnormally increased glucose oxidation in the failing heart. Our results indicate that the shift to ketone body metabolism during development of HF is an adaptive response. Strategies aimed at increasing ketone delivery to the heart could be useful in the treatment of early stages of HF.

#### **40. The taste of transcription: nuclear-localized bitter taste receptors activate calcium signaling and CREB phosphorylation**

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Taste family type 2 receptors (T2Rs) are G-protein coupled receptors (GPCRs) involved in bitter taste. T2Rs are also expressed outside the tongue, where they function in diverse roles. In upper-airway epithelia, T2Rs are localized to the plasma membrane and function to detect bitter bacterial quorum-sensing molecules, thereby activating innate immune responses. However, through both immunofluorescence and immunoblotting, we have found that many endogenously expressed T2Rs of the lower-airway epithelia are localized to the nucleus. Nuclear GPCRs are known to be important in some cell types, but nuclear GPCR signaling is not well studied. Many bitter compounds, including bacterial quorum-sensing molecules, are hydrophobic and likely cell permeant; thus, some bitterants may function to activate nuclear-localized T2Rs. Using genetically encoded nuclear-targeted fluorescent indicators, we found that some T2R agonists elevate nuclear calcium and are sensitive to PLC inhibitor U73122, consistent with canonical taste signaling ( $G\alpha$  gustducin and  $G\beta\gamma$  activation of phospholipase C). The known calcium-sensitive transcription factor CREB (cAMP response element-binding protein) is activated by phosphorylation from kinases such as protein kinase A or  $Ca^{2+}$ /calmodulin-dependent protein kinases. Here, we demonstrate that bitterants induce nuclear calcium release and activate CREB in lower airway epithelial cells.

#### **41. Functional organization of the islands of calleja in the olfactory tubercle**

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The Islands of Calleja (IC) are dense cell clusters located in the ventral striatum, predominantly in the olfactory tubercle (OT), an olfactory cortical region interconnected with brain reward centers. However, the functional properties and synaptic organization of the IC neurons are largely unknown. Unlike the ventral striatum, which mainly contains medium spiny neurons (MSNs) expressing dopamine D1 or D2 receptor, the IC contain tightly packed GABAergic granule cells expressing D3 receptor. By crossing transgenic D3-Cre mice with Cre-dependent reporter lines we obtained D3-tdTomato and D3-ChR2 (channelrhodopsin-2) mice for whole-cell patch clamp recording and optogenetic manipulation of the IC neurons. Based on the electrophysiological properties the IC neurons can be classified into two subtypes. Upon current injections, ~70% (39 out of 55) of the cells fired multiple spikes while the remaining 30% fired only a single spike. Further voltage-clamp analysis revealed that only the multiple spiking neurons exhibited predominant transient potassium current ( $I_A$ ) — fast inactivation of  $I_A$  may allow these neurons to fire repeatedly. We next examined local synaptic connections of the IC neurons. Upon blue light activation of the IC D3-ChR2 neurons, inhibitory postsynaptic currents were evoked in 66.7% (24 out of 36) of the OT MSNs and the majority (22 cells) received monosynaptic connection. Conversely, nearly half of the IC neurons (13 out of 29) received monosynaptic inhibition from the OT D1 MSNs. In addition, we have obtained the whole-brain projections from the IC D3-tdTomato neurons using CLARITY. We are currently investigating the functional significance of the IC neurons via genetic manipulation and mouse behaviors.

## 42. Social media mining for birth defect research

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Birth defects are the leading cause of infant mortality in the United States, but methods for studying birth defects remain limited. The primary objective of this study was to assess whether social media mining could be used to observe pregnancies with birth defect outcomes. We mined 432 million tweets posted by 112,647 users who publicly announced a pregnancy on Twitter. To retrieve sparse tweets that mention birth defects, we developed a bootstrapping approach that relies on lexicon, lexical variants, regular expressions, post-processing, and distributional properties. Inclusion criteria at the user level were verified by tweets indicating that the user's child had a birth defect, and accessibility to the user's timeline—all tweets posted by the user over time—during pregnancy. We conducted a semi-automatic evaluation to estimate the recall of our approach, and assessed the prevalence of selected birth defects reported on Twitter. We manually annotated 16,822 retrieved tweets, distinguishing ones indicating that the user's child (possibly) had a birth defect (true positives) from ones that merely mention birth defects (false positives). Inter-annotator agreement was  $\kappa = 0.79$  (Cohen's kappa). We inspected the timelines of the 646 users who posted true positives, and found 195 users who met the inclusion criteria. Congenital heart defects were the most common type of birth defect reported on Twitter, consistent with the general population. Based on an evaluation of an additional 4,169 tweets retrieved using alternative text mining methods, the recall of the approach was 0.95. Our results suggest that social media mining can complement existing methods of birth defect research by enabling a rare opportunity of observing pregnancies with birth defect outcomes.

### **43. A newfound role for *N*-acetylaspartate for preventing and reversing aggregation of amyloid-beta**

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Although *N*-acetylaspartate (NAA) has long been recognized as by far the most abundant amino acid in neurons, its primary role has remained a mystery. Based on its unique tertiary structure, we explored the potential of NAA to modulate the aggregation of amyloid-beta (A $\beta$ ) peptide 1-42 via multiple corroborating aggregation assays along with electron microscopy. Thioflavin-T fluorescence assays demonstrated that at physiological concentrations, NAA substantially inhibited the initiation of A $\beta$  fibril formation. In addition, NAA added after 25 minutes of A $\beta$  aggregation was shown to break up preformed fibrils. Electron microscopy analysis confirmed the absence of mature fibrils following NAA treatment. Furthermore, fluorescence correlation spectroscopy and dynamic light scattering measurements confirmed significant reductions in A $\beta$  fibril hydrodynamic radius following treatment with NAA. These results suggest that physiological levels of NAA could play an important role in controlling A $\beta$  aggregation *in vivo* where they are found in the same neuronal compartments.

#### **44. Childhood imprinting confers protection against H5N1 through hemagglutinin stalk-reactive antibodies**

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Influenza A viruses H1N1 and H3N2 circulate seasonally in humans. The avian IAV H5N1 has caused frequent epidemics in humans and represents a global pandemic threat. A recent epidemiological study showed that protection against severe disease from zoonotic H5N1 infection is closely associated with specific types of seasonal influenza virus strains that an individual likely encountered in childhood. Individuals who likely encountered H1N1 viruses in childhood were found to be protected against H5N1, whereas individuals that likely encountered H3N2 viruses in childhood were found to be susceptible to H5N1. Here, we performed a serological survey to measure the levels of neutralizing antibodies against H5N1 in 204 healthy adults. We found that individuals that have likely encountered H1N1 viruses during childhood have higher levels of neutralizing antibodies against H5N1. We hypothesized that the cross-reactive antibodies that neutralized H5N1 target the conserved HA stalk of H5N1. To investigate this, we measured the levels of stalk-reactive antibodies in these healthy adults and found that the amount of H1 stalk-binding antibodies correlated with neutralizing antibodies against H5N1. These studies suggest that early childhood H1N1 infections elicit long-lived antibody responses that can protect against pandemic influenza virus strains with similar HA stalk domains.

## 45. X-ray trigger sensitive biodegradable gold nanoparticles as imaging and therapeutic agents

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Gold nanoparticles (AuNP) are well established contrast agents owing to their optical properties, high X-ray attenuation, and biocompatibility. The size-dependent excretion profile of NPs has recently motivated the development of small and excretable AuNP-loaded polymeric nanoparticles, designed to break down through hydrolysis for AuNP excretion. However, “on-demand” spatiotemporal breakdown of such NPs would be highly desirable to trigger agent excretion. While common triggers such as light close to the visible range or ultrasound have low tissue penetration that limits their use, X-rays have no penetration limit and can be administered in a highly focused fashion. Therefore, we developed novel nanoparticles whose breakdown can be triggered by X-rays to promote fast excretion of AuNP within the body. Towards this goal, we combined biodegradable poly-di(carboxylatophenoxy)phosphazene (PCPP) with the X-ray sensitive polymer hyaluronic acid (HA), and loaded them with high payloads of small (~4 nm) AuNPs. These nanoparticles have a diameter of  $255.5 \pm 64.4$  nm from transmission electron microscopy (TEM) measurements and the presence of both AuNP and HA was confirmed by inductively coupled plasma optical emission spectrometry (ICP-OES) and Fourier transform infrared spectroscopy (FT-IR), respectively. Good biocompatibility of Au-PCPP/HA NPs were observed using the LIVE-DEAD assay on three-cell lines – HepG2, SVEC4, and MDA-MB-231 – incubated for 8 hours with cell medium treated with Au-PCPP/HA NP at up to 1 mg/mL. The X-ray triggered degradation of Au-PCPP/HA NPs suspended in PBS-10% FBS at various doses from 5 Gy to 60 Gy promoted a gold release of over 50% of the encapsulated AuNPs over 7 days. Finally, the formulation was adapted to incorporate fluorescein-labeled bovine serum albumin (FITC-BSA) along with AuNP as a proof-of-concept for X-ray targeted delivery of a therapeutic protein.

#### 46. A novel mouse model with *Tubb4a*<sup>D249N/D249N</sup> for classical hypomyelination and atrophy of basal ganglia and cerebellum

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Hypomyelination and atrophy of basal ganglia and cerebellum (H-ABC) is a hypomyelinating leukodystrophy most frequently instigated by a point mutation of p.Asp249Asn (D249N), which occurs as a heterozygous mutation in the tubulin alpha 4 (*TUBB4A*) gene. H-ABC onset begins in early childhood and is characterized with dystonia, ataxia, altered gait, and progressive motor dysfunctions. To date, there is no cure and no therapeutic approach available for the disease. To understand how the *Tubb4a* mutation causes H-ABC and to facilitate the development of therapeutic strategy, we developed a knock-in mouse model harboring heterozygous (*Tubb4a*<sup>D249N/-</sup>) or homozygous (*Tubb4a*<sup>D249N/D249N</sup>) mutation using a CRISPR-Cas9 approach. We report this as the first mouse model of classical H-ABC (*Tubb4a*<sup>D249N/D249N</sup>), which displays decreased survival, progressive motor dysfunction and tremulous behavior, abnormal gait, and ataxia, thus recapitulating the phenotypic features of the disease. Neuropathological assessment of *Tubb4a*<sup>D249N/D249N</sup> mice on post-natal day 21 and end-stage post-natal day 40 showed decreased PLP staining in the corpus callosum and drastic loss of neurons in the striatum and cerebellum. Ultrathin brain sections for electron microscopy further revealed a severe loss of myelin in the spinal cord and optic nerve of these mice. It is not known whether hypomyelination resulted from the impairment of oligodendrocyte (myelin forming cells in central nervous system) maturation or survival. Ongoing studies are focused on understanding the mechanism of *Tubb4a* mutation-mediated effects with respect to oligodendrocyte and neurons. The *Tubb4a*<sup>D249N/D249N</sup> mice provide a novel mouse model for H-ABC, which would help target therapeutic pathways that preserve myelin, prevent neurodegeneration, and increase the survival of H-ABC patients.

#### **47. Development of a novel syngeneic model for ovarian cancer research**

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Ovarian cancer is the most lethal gynecologic malignancy and afflicts nearly a quarter of a million women each year worldwide. High-grade serous ovarian carcinoma (HGSOC) is the most common subtype and most are thought to arise from fallopian tube (FT) secretory epithelial cells. Although a number of human models have been developed, no murine FT-derived cancer cell lines are available for ovarian cancer research. The most frequently used murine cancer cell line is the ID8 model, derived from the ovarian surface epithelium of a purebred C57Bl6 mouse. Recent genomic characterization of ID8 revealed that this line does not harbor the hallmarks of human HGSOC. Thus, we hypothesize that developing a murine FT-derived cell line that recapitulates the phenotypic and genomic hallmarks of human FT epithelial cells and HGSOC, and will enable studies in immunocompetent animal, thereby addressing novel therapeutic combinations, especially immunotherapies. We have established and immortalized a C57Bl/6 murine oviductal primary secretory epithelial cell line by using the SV40 large T antigen, which binds and inactivates the p53 and pRb tumor suppressor proteins. The immortalized C57Bl/6 murine oviductal cells were then further transformed by deleting genes typically altered in human HGSOC (*Brca1* or *Brca2*, and *Pten*). Successful transformation of oviductal cells was monitored *in vitro* using proliferation and clonogenic assays as well as anchorage-independent growth capability. Tumorigenicity *in vivo* was assessed by implanting cells into syngeneic mice. By developing transformed oviductal cells from purebred C57Bl6 mice, we will be able to assess the immune component of these tumors and determine how different tumor genetics influence immune infiltrates in a syngeneic system.

#### **48. MIRO-1 determines mitochondrial shape transition upon GPCR activation and Ca<sup>2+</sup> stress**

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Mitochondria shape cytosolic calcium ([Ca<sup>2+</sup>]<sub>c</sub>) transients and utilize the mitochondrial Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>m</sub>) in exchange for bioenergetics output. Conversely, dysregulated [Ca<sup>2+</sup>]<sub>c</sub> causes [Ca<sup>2+</sup>]<sub>m</sub> overload and induces permeability transition pore and cell death. Ablation of MCU-mediated Ca<sup>2+</sup> uptake exhibited elevated [Ca<sup>2+</sup>]<sub>c</sub> and failed to prevent stress-induced cell death. The mechanisms for these effects remain elusive. Here, we report that mitochondria undergo a cytosolic Ca<sup>2+</sup>-induced shape change that is distinct from mitochondrial fission and swelling. [Ca<sup>2+</sup>]<sub>c</sub> elevation, but not MCU-mediated Ca<sup>2+</sup> uptake, appears to be essential for the process we term mitochondrial shape transition (MiST). MiST is mediated by the mitochondrial protein Miro1 through its EF-hand domain 1 in multiple cell types. Moreover, Ca<sup>2+</sup>-dependent disruption of Miro1/KIF5B/tubulin complex is determined by Miro1 EF1 domain. Functionally, Miro1-dependent MiST is essential for autophagy/mitophagy that is attenuated in Miro1 EF1 mutants. Thus, Miro1 is a cytosolic Ca<sup>2+</sup> sensor that decodes metazoan Ca<sup>2+</sup> signals as MiST.

#### **49. Alteration of neuroblastoma tumor microenvironment by polyamine blockade**

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Neuroblastomas (NBs) are oncogenically driven by amplification of *MYCN*. Myc controls the transcription of the rate-limiting enzyme in polyamine synthesis, ornithine decarboxylase (Odc1), and NBs contain high levels of polyamines. Odc1 is irreversibly inhibited by difluoromethylornithine (DFMO), and in vitro exposure of NB cells to this drug results in modest growth retardation. In a mouse model of MYCN-driven NB, administration of DFMO significantly extends survival. We therefore hypothesized that, in addition to its NB-intrinsic effects, DFMO also alters the tumor microenvironment (TME). Indeed we find that DFMO exposure induces an increase in the frequency of intratumoral NK cells. Furthermore, while intratumoral (but not splenic) NK cells from untreated mice appear dysfunctional, DFMO exposure appears to mitigate this, and the DFMO exposed NK cells appear to be poised to kill neuroblasts. Additionally, we demonstrate that these effects are not due to the antimicrobial effect of DFMO, as no significant changes in the intestinal microbiota were observed in DFMO-exposed mice. Interestingly, both treated and untreated mice display a larger than expected frequency of invariant natural killer T (iNKT) cells; these cells possess antitumor cytotoxic activity and also robustly secrete pro-inflammatory cytokines that promote the antitumor responses of other effector lymphocytes including NK cells. Indeed, higher frequencies of intratumoral iNKT cells are associated with increased survival in NB. Using proof-of-concept reagents, we demonstrate the ability to redirect iNKT cells to kill NB targets in vitro and to activate intratumoral iNKT cells. We are currently investigating whether the use of such reagents could synergize with DFMO to induce sustained control of NB growth.

## 50. Characterization of protein arginylation by mass spectrometry

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Arginylation is a tRNA-mediated process known only to be catalyzed by the arginyltransferase enzyme ATE1, or its related splice isoforms, and involves the post-translational addition of arginine to both protein or polypeptide N-termini and internal acidic residue sidechains. Additionally, *Ate1*-KO cells are known to form subcutaneous tumors in immunocompromised mouse xenograft models and exhibit cancerous phenotypes at the cellular level, rendering *Ate1* a candidate tumor suppressor gene. This is also supported by the observation that ATE1 protein levels are down-regulated in a number of human cancers and the inverse correlation of its level of transcription with metastatic progression. Despite *Ate1* being an essential gene in higher eukaryotes, the pervasiveness and regulatory impact of protein arginylation remains largely enigmatic due to the lack of dedicated tools geared toward its study. We will discuss progress and ongoing efforts toward developing a dedicated mass spectrometry-based proteomic approach for the identification of arginylated proteins in cells in order to further understand the role of arginylation in routine cellular function and disease.

## 51. $\beta$ -arrestin2 differentially regulates MrgprB2-induced pseudo-allergy and Fc $\epsilon$ RI-mediated cutaneous allergic reactions

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Murine mast cells express Mas-related G protein couple receptor B2 (MrgprB2) and high affinity IgE receptor (Fc $\epsilon$ RI)<sup>1-2</sup>. Mast cell degranulation by drugs via MrgprB2 leads to pseudoallergic reactions but their activation via Fc $\epsilon$ RI results in early anaphylactic and delayed hypersensitivity reactions<sup>3</sup>.  $\beta$ -arrestin 2 ( $\beta$ -arr2) regulates GPCR signaling but its effect on MrgprB2 and Fc $\epsilon$ RI-mediated responses in MCs have not been determined<sup>4</sup>.

**Methods:** Peritoneal mast cells (PMCs) were used to determine the effect of  $\beta$ -arr2 on ciprofloxacin-induced degranulation *ex vivo* and pseudoallergic drug reaction *in vivo*. Antigen/IgE-mediated signaling, degranulation and chemotaxis were performed in wild-type and  $\beta$ -arr2<sup>-/-</sup> bone marrow-derived MCs. The role of  $\beta$ -arr2 on early and delayed hypersensitivity reactions were investigated in IgE-mediated passive cutaneous anaphylaxis (PCA) and trimelitic anhydride (TMA)-induced skin inflammation, respectively.

**Results:** Ciprofloxacin induced significantly higher degranulation in  $\beta$ -arr2<sup>-/-</sup> PMCs and induced greater pseudoallergic skin reaction in  $\beta$ -arr2<sup>-/-</sup> mice than in wild-type mice. Absence of  $\beta$ -arr2 in BMMCs had no effect on antigen/IgE-mediated Syk phosphorylation, Ca<sup>2+</sup> mobilization but resulted in both enhanced degranulation *ex vivo* and PCA reaction *in vivo*. By contrast,  $\beta$ -arr2 deficiency resulted in significant reduction in both antigen/IgE-mediated chemotaxis in BMMCs and TMA-induced skin inflammation in mice.

**Conclusions:**  $\beta$ -arr2 downregulates acute pseudo-allergic and allergic reactions by inhibiting MC degranulation but enhances chronic allergic hypersensitivity reactions by promoting MC chemotaxis. Thus,  $\beta$ -arr2 may serve as an important target for modulating MC-mediated pseudoallergy/allergic responses.

## 52. Comprehensive molecular and functional characterization of ovarian clear cell carcinoma cell lines for drug development

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Ovarian cancer is a heterogeneous disease. While high-grade serous carcinoma (HGSC) is the most common, clear cell carcinoma (CCC) is notoriously the most challenging to treat. To improve the survival of patients with ovarian CCC, a deeper understanding of the molecular features of available model systems is needed. Our goal is to characterize a panel of CCC lines genomically and functionally and identify those that can serve as tractable model systems for future drug discovery studies. **Methods:** We characterize 9 CCC cell lines (ES-2, TOV21G, OVTOKO, OVMANA, OCI-C5x, JHOC-5, JHOC-7, JHOC-9, and OVISE) with whole genome sequence and proteomics approaches (reverse phase protein array; RPPA). We also test *in vivo* tumorigenic potential, by injecting 5 million cells of luciferized CCC lines in NSG female mice subcutaneously or intraperitoneally. **Results:** Among the 9 CCC lines, *ARID1A* and *PIK3CA* mutation were detected in 7 and 4 cell lines, respectively. The ES-2 line has *TP53* and *BRAF* mutation and its genomic profile is inconsistent with CCC. Principal component analysis of RPPA showed distinct clusters between the 9 CCC lines and 6 HGSC lines. Interestingly, we observed two distinct clusters within the CCC lines. Consistent with our genomic analysis, ES-2 correlated with HGSC lines in RPPA. In xenograft study, 4 cell lines (ES-2, TOV21G, OVTOKO, and OCI-C5x) formed tumor within a month, suggesting they are useful tools for *in vivo* studies. **Conclusions:** Our genomic studies identified aberrations in CCC lines not previously described. We identified 4 cell lines that readily form tumors in mice and could be used for future *in vivo* studies. Interestingly, ES-2 appears to cluster more closely with HGSC and may not represent the CCC histotype.

### **53. Targeting conformational entropy to modulate binding affinity**

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Molecular recognition by proteins is fundamental to biology. Enthalpy, the hydrophobic effect and specific interactions at the interface are often assumed to govern binding energetics, but alone cannot explain the observed consequences of ligand binding. Without knowledge of protein entropy, the thermodynamic picture of molecular recognition is incomplete. Recent developments in nuclear magnetic resonance spectroscopy (NMR) have provided an experimental way to quantify the contribution from protein entropy to binding<sup>1</sup>. We apply this approach to barnase-barstar, one of the strongest protein-protein interactions known. Both proteins become dynamically more rigid upon binding, indicative of a large entropic penalty. Our strategy was to attempt to diminish this entropic penalty by introducing an intra-molecular disulfide bond far from the interface to make the bound and unbound states more similar. We characterized the variant protein structurally (X-ray crystallography, NMR spectroscopy), dynamically (NMR relaxation), thermodynamically (ITC, DSC), and kinetically (stopped-flow). The results indicate that the engineered disulfide produces no significant structural changes and only small changes in the enthalpy of binding. Instead, the disulfide quenches the unfavorable change in side chain dynamics and a concomitant increase in the binding free energy is observed that pushes the affinity of the complex beyond fM. Kinetics measurements reveal that this tighter complex results mostly from an accelerated on-rate. Our work illustrates how protein entropy is a fundamental determinant of molecular recognition and a variable that can be manipulated to alter the affinity of complexes. Supported by the NIH and the Mathers Foundation.

#### **54. Mitochondrial function is compromised by deletion of YY1 in B cells stimulated through BCR, but not TLR pathways**

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The ubiquitously expressed transcription factor Yin Yang 1 (YY1) plays a vital role in major biological processes including gene expression, proliferation, development, and differentiation. YY1 activates or represses transcription by binding to promoters or enhancers, and recruiting specific co-factors to DNA. YY1 function in long-distance DNA interactions is also well established, and YY1 plays a critical role in controlling long-distance DNA loops needed for immunoglobulin class switch recombination and V(D)J somatic rearrangement. YY1 has also been suggested to control genes important for mitochondrial function. Therefore, we tested the impact of YY1 conditional deletion on mitochondrial activities. We found that after conditional deletion of YY1 in primary splenic B cells followed by activation with anti-IgM, there was a significant loss of mitochondrial mass and potential. This corresponded with mitochondrial structural changes, a drop in metabolic oxygen rate consumption, and greater dependence on glycolysis. These changes were not observed in cells stimulated with LPS plus IL4. RNA-seq analyses showed many differentially regulated genes involved in mitochondrial function after conditional deletion of YY1, and ChIP-seq studies indicated that many of these gene promoters directly bound to YY1. We evaluated the mitochondrial-related genes differentially regulated by YY1 after stimulation with either anti-IgM compared to LPS plus IL4, and propose that the dramatic change in mitochondrial function after deletion of YY1 and stimulation with anti-IgM is due to mis-regulation of several genes involved in oxidative phosphorylation.

## **55. Providers' understandings of uncertainty in genetic testing for inherited cardiac disease**

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Most prior bioethics research on uncertainty in genetics and genomics has focused on how patients and families receive and interpret genetic information. It is equally important to examine clinicians' understandings of uncertainty in genetics because they often serve as gatekeepers to genetic testing, and because it is becoming more common for practitioners without specific expertise in genetics/genomics to order genetic testing for their patients. In my research, I explore clinicians' experiences ordering genetic testing for inherited cardiac disease. Of the genes determined to be "clinically actionable" by the American College of Medical Genetics and Genomics, half are related to cardiac disease. But there is still disagreement within the field about which types of patients should be tested, and when subsequent intervention is appropriate. I focus specifically on how clinicians navigate the tension between identifying real disease risks for their patients and concerns about overtreatment. Based on semi-structured, in-depth interviews with clinical geneticists, physicians, and genetic counselors, I find that there is considerable variability in the ways that clinicians determine which patients are good candidates for genetic testing, and how they understand the uncertainty of genetic testing. Variability is particularly high when considering patients with no physical symptoms of a disease. Based on these findings, I provide a theoretical framework to help practitioners and bioethicists understand how and when genetic information can be both helpful and hurtful in managing patient health.

## 56. Defining the broad posttranscriptional regulatory functions of Esrp1

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The epithelial splicing regulatory proteins, Esrp1 and Esrp2, are cell type specific RNA binding proteins that are involved in maintaining epithelial cell function, primarily through splicing regulation. Investigations into the functional roles of Esrp proteins revealed a splicing network required for expression of epithelial specific isoforms of genes that play roles in cell adhesion, polarity, and cytoskeletal organization. Although Esrp1 and Esrp2 share partial functional redundancy, Esrp1 appears to play a larger role in regulating gene expression. Esrp1 isoforms have variable inclusion of a nuclear localization signal leading to expression of protein isoforms with distinct nuclear and cytoplasmic localization. We hypothesize that Esrp1 has distinct cytoplasmic functions that work in concert with splicing regulation in the nucleus to promote epithelial cell differentiation and function. To study the distinct roles of Esrp1, we use crosslinking immunoprecipitation (CLIP) to determine global Esrp1 protein-RNA interactions in tissues with robust Esrp1 expression to identify direct targets. Using CLIP, we identified the direct targets of Esrp1 *in vivo* and complemented previous *in vitro* data, which determined the RNA binding motif and specificity of Esrp1. Moreover, we use the data to infer cytoplasmic functions for Esrp1. Collectively, my research will elucidate the roles of isoform specific ESRP1 by deciphering the contribution of the cytoplasmic and nuclear isoforms of ESRP1 in the epithelial posttranscriptional gene regulatory network.

## 57. Ndr kinases regulate amacrine cells proliferation and homeostasis

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*Ndr2/Stk38l* encodes a protein kinase associated with the Hippo tumor suppressor pathway. Recent studies suggest that Ndr2 protein kinase is important for retina maintenance, although its precise retinal functions are unknown. Notably, an Ndr2 loss-of-function allele causes early retina degeneration in dogs, characterized by opsin mislocalization and concurrent increase in photoreceptor apoptosis and proliferation. Separate studies indicate that Ndr2 and its paralog Ndr1/Stk38 regulate cell proliferation, morphogenesis and gene expression in other tissues. To elucidate the retinal functions of Ndr2 and Ndr1, we generated Ndr1 and Ndr2 single knockout mice and characterized their retinal phenotypes by immunofluorescence microscopy, immunoblotting and gene expression analysis. Although retinal lamination appeared normal in these mice, Ndr deletion caused a subset of Pax6-positive amacrine cells to proliferate in differentiated retinas, while concurrently decreasing the number of GABAergic and Pax6-positive amacrine cells. Retinal transcriptome analyses revealed that Ndr2 deletion increased expression of neuronal stress genes and decreased expression of synaptic organization genes. Consistent with the latter, Ndr deletion dramatically reduced levels of Aak1, an Ndr substrate that regulates vesicle trafficking. Our findings indicate that Ndr kinases are important regulators of amacrine and photoreceptor cells and suggest that Ndr kinases inhibit the proliferation of a subset of terminally differentiated cells and modulate interneuron synapse function via Aak1. Further analyses of retinal NDR mechanisms may influence the development of therapeutic interventions for retinal degenerations and methods to stimulate photoreceptor regeneration.

## 58. Differential performance of polygenic risk scores across populations

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Most genome-wide association studies (GWAS) are performed in cohorts of European ancestry (EA). Differences in polygenic risk scores (PRS) between EA and non-EA (NEA) ancestry populations are believed to be largely spurious. However, there has thus far been little quantitative investigation into the accuracy of applying EA PRS between populations and the feasibility of using EA PRS on NEA groups. To test this issue, our study is exploring the prediction accuracy of PRS for a highly polygenic trait: height. We computed PRS from independent SNPs based on 60 different clumping/pruning methods, and compared PRS based on effect sizes from GWAS performed in individuals of EA from GIANT and the UK Biobank (UKB). Like others, we found average differences in PRS based on population. However, we also find that the magnitude depends on the source of the summary statistics, and the clumping strategy employed indicating that the differences between populations are not biologically meaningful. For example, the difference between PRS for EA and African ancestry (AA) individuals varies from 0.83-6.77 standard deviations (SD) across clumping methods on GIANT and 0.62-2.31 SD on UKB between databases. Using UKB effect sizes for individual-level prediction in ~7,300 AA and EA individuals, we find that PRS explains up to ~4.24% and 11.3% of height variation, respectively, and this percentage is significantly positively correlated with the proportion of an individual's EA. Interestingly, the correlation between height and EA is extremely low and genome-wide ancestry explains only 0.05% of the variance in height, confirming that cross-population differences in PRS do not correlate to phenotypic differences.

## **59. Sigma-2 Receptor and PGRMC1 increase internalization of LDL by LDL Receptor through the formation of a ternary complex**

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The Sigma-2 Receptor (TMEM97) and Progesterone Receptor Membrane Component 1 (PGRMC1) have been implicated in cholesterol homeostasis. Both are widely expressed in the nervous system and regulate signaling pathways important to neurodegenerative diseases. Their involvement in trafficking, cholesterol homeostasis pathways, presence on membranes suggest an involvement with the Low-Density Lipoprotein Receptor LDLR mediated uptake of LDL. Therefore, the hypothesis of this study is that the interaction of both TMEM97 and PGRMC1 with LDLR are important for uptake of LDL via the formation of a complex with LDLR, facilitating its successful internalization. HeLa cells were utilized as a model system, CRISPR/Cas9 was used to generate knockouts of TMEM97, PGRMC1, and TMEM97/PGRMC1 double knockout (DKO) cell lines. This was used to assess the effect these receptors have on uptake of LDL radiolabeled with [<sup>3</sup>H]cholesterol and the localization of the proteins. Ablation of one or both of the receptors result in similar inhibition of LDL uptake, suggesting the presence of both TMEM97 and PGRMC1 are necessary for the internalization of LDL-LDLR complex. Knockout cells did not show a reduction of LDLR levels, nor a reduction in its capacity to bind LDL, suggesting their involvement in the internalization process. Treating control cells with a TMEM97 ligand similarly decreased LDL uptake. Immunofluorescence indicated that PGRMC1 and LDLR colocalize on the plasma membrane, and upon treatment with LDL, TMEM97 becomes associated with the complex. Taken together TMEM97 and PGRMC1 are involved in internalization of LDL by interacting with the LDLR upon LDL binding. The interaction of all three receptors are necessary for lipoprotein uptake via formation of a protein complex required for internalization.

## **60. Understanding and modulating expression of developmentally regulated RNA binding protein CELF2**

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Members of the CELF (CUGBP, ELAV-like Family) are RNA binding proteins known to regulate splicing transitions that directly influence cardiac remodeling and cytoskeletal rearrangement. One of the CELF splicing regulators – CELF2 – has been linked to myotonic and muscular dystrophies. However, many aspects of CELF2 expression in cardiac and muscle tissues are unknown. Previously, we have shown that regulation of CELF2 in T-cells is due in large part to c-Jun N-terminal Kinase (JNK)-dependent control of the CELF2 mRNA stability. This stabilization correlates with a dramatic change in CELF2 3' untranslated region (3'UTR) during both T-cell and cardiac development. Given the conservation of developmentally-induced 3'UTR regulation of CELF2 across tissues, the goal of my research is to understand the mechanism and regulation of CELF2 3'UTR expression and identity in both Jurkat T-cells and C2C12 cells. To understand the causal relationship of CELF2 3'UTR identity and CELF2 expression, I used quantitative reporter gene assays and CRISPR-Cas9 mediated modifications of the 3'UTR. My results indicated that retention of the 3'UTR intron limits protein expression, suggesting a model in which increased 3'UTR intron retention upon T-cell or myotube differentiation serves to prevent excessive protein expression. Consistently, I show that CELF2 protein controls alternative events of the 3'UTR. Strikingly, the distinct 3'UTR isoforms show essentially identical abundance of mRNA, suggesting that the expression of these 3'UTR sequences is translationally regulated. Further, I show that stability of 3'UTR isoforms are similar, but depend on JNK-signaling. Next, I will study if JNK regulates the expression of stabilizing proteins or destabilizing proteins to influence CELF2 mRNA stability.

## **61. Determining the stages of transcriptomic maturation of human fetal and hPSC-derived beta cells**

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Diabetes is rapidly becoming a global epidemic, with a staggering health, societal, and economic impact. The cure for Type 1 Diabetes (T1D) has remained elusive. Although promising, cadaveric islet transplantation is limited by the availability of donor islets. Guided differentiation of human pluripotent stem cells (hPSC) to transplantable beta-like cells requires a detailed understanding of pancreas development and beta cell maturation. Single-Cell RNA-Sequencing (scRNA-seq) allows for the interrogation of transcriptional changes at the single cell level. It is particularly well suited for analyzing heterogeneous cell populations during development. We hypothesized that human fetal beta cells undergo a stepwise transcriptomic maturation that is not completed in current in vitro stem cell differentiation protocols. We isolated single pancreatic cells from human fetal pancreas at mid-gestation and hPSC-derived beta-like cells. We utilized 10x Chromium platform to generate scRNA-seq libraries for 897 fetal pancreatic endocrine cells and 5,454 hPSC-derived beta-like cells and compared them with the transcriptomes of 6,241 adult human beta cells. We then aimed to reconstruct a developmental trajectory of fetal, adult, and hPSC-derived beta cells. By ordering cells along a pseudotemporal trajectory of differentiation, based on transcriptional similarities, we aim to create a continuum of beta cell transcriptomic maturation and characterize beta cell specific maturational markers. Thus, we can narrow the existing knowledge gap between fetal and hPSC-derived beta-like cells, and mature adult beta cells. The results of this research will benefit the improvements of in vitro beta cell differentiation protocols in providing a cell replacement therapy for T1D patients.

## **62. Rare mutations on the cystic fibrosis transmembrane conductance regulator's extracellular face inhibit biogenesis**

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There are over 2,000 mutations in the Cystic Fibrosis Conductance Regulator (CFTR) gene, most causing CF. Only a minority of these mutations are found on the extracellular face. Our group has demonstrated a novel ER lumen chaperone, ER protein of 29kDa (ERp29), has increased expression in response to 4-PBA treatment, promotes normal biogenesis of wtCFTR and corrects aberrant F508del when overexpressed. ERp29 must interact with CFTR on the ER-luminal face of the protein, F508del is located on the CFTR's cytoplasmic face. Other trafficking components such as coat complex II mediate CFTR's exit from the ER to the Golgi, also interact with CFTR's cytoplasmic face. ERp29 is suggested to interact with  $-(F, Y)-X-(F, Y)-$  motif on client proteins, and there are 2 rare CF mutations occurring in this motif, Y1014C and F1016S. This motif occurs within the extracellular loop 5 (ECL5). We hypothesize that these mutations will cause abnormal channel biogenesis. In addition, there are 2 other rare mutations adjacent to this motif at proline 1013 (P1013H and P1013L), which are likely to change the structure of the ECL5. To test the hypothesis that ECL5 mutations inhibit CFTR biogenesis, we expressed mutant CFTRs in CFBE41o<sup>-</sup> CF cells. Functional expression of mutant CFTR, defined as  $I_{sc}$  that was inhibited by apical application of 10  $\mu$  M CFTRinh-172 after treatment of the cells with 10  $\mu$  M forskolin and 100  $\mu$  M IBMX and imposition of the basolateral-to-apical chloride gradient in Ussing chambers, was absent. Immunoblots of whole cell lysates of CFBE41o<sup>-</sup> transfected with these mutants demonstrated CFTRs that co-migrated with F508del CFTR and at a lower MW than wtCFTR. These data support the hypothesis that CF causing CFTR mutations in ECL5 and on CFTRs luminal face inhibit CFTR biogenesis.

### **63. Adenovirus co-opts host factors to mediate m6A modification of viral RNAs important for splicing and protein production**

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The discovery of reversible N6-methyladenosine (m6A) modification of RNA has fundamentally altered our view of the central dogma of molecular biology. The m6A chemical modification is added post-transcriptionally to RNA, where it has been implicated in such diverse processes as RNA splicing, nuclear export, stability, and translation. While it is known that Adenovirus RNAs are marked by m6A, the effect of this modification has never been deciphered. Using methylated RNA Immunoprecipitation and Sequencing (meRIP-Seq), we have identified the specific locations of m6A modifications within the Adenovirus transcriptome. Every major transcriptional unit contains at least one m6A peak, and these peaks are enriched near the 3' splice sites of the heavily spliced Adenovirus major late transcriptional unit. Furthermore, we have shown that during infection Adenovirus recruits host factors involved in methylating RNA and binding methylated RNA to sites of active viral transcription. Knockdown or knockout of the human m6A-specific methyltransferase complex reduces viral late gene expression, protein production, and the production of infectious virus particles. In addition, loss of m6A function reduces the splicing efficiency of adenoviral late genes, while sparing early gene transcription and splicing. This result is phenocopied by knockdown of cellular YTHDC1, a known m6A-binding protein that associates with splicing factors. These data demonstrate that m6A modification of viral RNA is important during the late stage of Adenoviral infection. These experiments are among the first to show how a DNA virus co-opts cellular epitranscriptomic machinery to mediate viral RNA biogenesis. Furthermore, these findings further validate the role of m6A in splicing of RNA transcripts.

#### **64. Hypocretin/orexin in rostromedial tegmental nucleus: neuroanatomy and functional involvement in a rat model of cocaine addiction**

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Addiction to psychostimulants like cocaine negatively influences people across the globe including in the US. Psychostimulants act on cells producing dopamine (DA) within the ventral tegmental area (VTA) by augmenting transmission to the forebrain. However, the neural circuitry regulating DA release is still being clarified. For example, hypothalamic neurons that produce hypocretin/orexin (hcrt/ox) innervate the VTA and regulate motivated behaviors. Recently, the caudal portion of the VTA, called the rostromedial tegmental nucleus (RMTg), was shown to negatively regulate VTA DA and contribute to processing aversive stimuli. While the hypothalamus provides input to RMTg, cell-type identities and potential functions of this neural circuit are not known. Here, we mapped the hcrt/ox-RMTg projections and assessed the effects of hcrt/ox transmission in RMTg on motivation and affect in a model of cocaine abuse. Rats (n=4) received unilateral retrograde tracer deposits in RMTg, and tracer-containing hcrt/ox cells were quantified. Results show that hcrt/ox neurons project to RMTg at a comparable density ( $6.7 \pm 2.2\%$ ) relative to VTA projections ( $8.0 \pm 2.7\%$ ). In a separate cohort, rats (n=7) were trained to self-administer cocaine (0.75 mg/kg/inf) prior to intra-RMTg microinjection of hcrt-1/ox-A (0.0 - 3.0 nmol/hemi). Ultrasonic vocalizations (USVs) were measured as an index of affective state with 22- and 50-kHz USVs reflecting negative and positive states. Intra-RMTg hcrt/ox suppressed motivated cocaine-taking without altering USV counts relative to vehicle-pretreated comparator sessions. Ongoing work is probing the ability of hcrt/ox to influence RMTg cell physiology which will shed insight into the circuitry contributing to the development of reward-related psychiatric conditions like addiction.

## 65. Mitochondrial targeted biofuels as countermeasures against chemical threats

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Chemical exposure due to warfare or chemical accidents poses a threat to the safety of civilians and military personnel. Many chemical agents have been implicated to impair mitochondrial function with mitochondrial complex I (CI) being most commonly affected, thereby reducing cellular ATP production. Complex II (CII) function often remains unaffected by these chemicals, presenting a possible treatment target. We are investigating cell-permeable prodrugs of the CII substrate succinate as potential treatment for chemically induced mitochondrial toxicity.

**Methods:** Mitochondrial respiration and lactate production of human platelets and primary human fibroblasts was evaluated *in vitro* after exposure to a variety of chemical agents and the ability of the cell-permeable prodrugs of succinate to improve the toxic phenotype was assessed.

**Results:** Chlorpyrifos (CPF) significantly reduced CI-linked mitochondrial respiration and induced increased lactate production over time. Sodium fluoroacetate (SF) impaired respiration upstream of CI. The cell-permeable succinate prodrugs rescued the respiratory defects induced by SF and the well-known complex IV inhibitor sodium azide. They further attenuated the CPF-induced increased lactate production.

**Conclusion:** The cell-permeable succinate prodrugs counteracted the energy deficit induced by CPF, SF and sodium azide *in vitro*. If similar effects prove translatable to an *in vivo* effect, this drug class presents a promising treatment strategy to improve the medical response capabilities in chemical emergencies. A pharmaceutical countermeasure improving the function of a common toxicological target rather than targeting a single chemical agent would be widely applicable as treatment against chemical threats.

## 66. In host adaptation of nontuberculous mycobacteria in a cystic fibrosis patient

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Nontuberculous mycobacteria (NTM) are detrimental CF pathogens with substantial challenges surrounding diagnosis and treatment but mechanisms by which NTM adapts to develop chronic infection are largely unknown.

**Research Rationale:** Longitudinal whole genome sequencing (WGS) can identify genetic determinants over time, because advantageous adaptations are represented by the most evolutionarily fit clades.

**Methods:** We obtained 24 NTM samples from a single patient (PT101) with chronic NTM infection over 5-years. DNA was extracted, WGS completed, and our bioinformatic pipeline employed for post-sequencing processing and analyses.

**Results:** All 24 isolates were identified as *M. avium* subsp. *hominissuis* (MAH). Genomes averaged 5.3 Mb (68.9% G+C). Temporally-related isolates were dispersed throughout distinct clades. Bayesian-inferred phylogeny suggested that the most recent common ancestor arose 12 years prior to NTM disease, suggesting an initial infection with a clonal complex or long-term chronic colonization. We identified hundreds of unique core genes in our isolates that were absent in all other publicly-available complete *M. avium* complex genomes, likely deriving from a large conjugative plasmid. We found 15 core genes with SNPs affecting protein-coding function by dN/dS analyses. Ancestral reconstructions noted time-ordered mutations these genes and important antibiotic resistance genes.

**Conclusions:** Chronic MAH infection in PT101 was characterized by long-term maintenance of population diversity with adaptive genotypic and phenotypic changes. Our approach demonstrated population dynamics of NTM, highlighted recombination as a likely adaptive evolutionary modality, and identified genetic changes that may enhance NTM fitness in chronic CF infections.

## 67. A role for the KDEL Receptor in ENaC trafficking

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ERp29 is a 29 kDa thioredoxin-homologous protein of the endoplasmic reticulum (ER) that displays chaperone-like properties, and its expression is increased by treatment with the F508del-CFTR corrector, 4-Phenylbutyrate. Previously we demonstrated that ERp29 promotes CFTR biogenesis (Suaud, et al., JBC 2011) and regulates ENaC biogenesis and functional expression (Grumbach, Bikard, et al., AJPCell, 2014). In this work, we found that overexpression of wt ERp29 increased the abundance of the active form of  $\gamma$ -ENaC, whereas overexpression of a mutant ERp29 (C157S ERp29) decreased ENaC functional expression. There was no altered expression of  $\beta$ -ENaC at the apical surface, suggesting that ERp29 may modulate ENaC open probability. ERp29 overexpression promoted the interaction of both ENaC and CFTR with the coat complex II (COP II) ER exit machinery, whereas C157S ERp29 overexpression decreased this interaction, suggesting a model where ERp29 may promote ENaC cleavage by directing ENaC to the Golgi. ERp29's C-terminal ER retention motif is KEEL, a KDEL variant associated with less robust ER retention. To test whether this motif is critical for ERp29's regulation of ENaC and CFTR, we designed mutant ERp29s (ERp29 KDEL and ERp29  $\Delta$ KEEL). ENaC functional expression decreased with expression of ERp29  $\Delta$ KEEL, but not with ERp29 KDEL.  $\beta$ -ENaC expression at the apical surface was not altered. We therefore tested the hypothesis that this was due to interaction with the KDEL Receptor (KDELRL1), and found that depletion of KDELRL1 decreased ENaC processing. Our findings suggest a role for ERp29's association with KDELRL1 in the biogenesis of CFTR and ENaC, and therefore suggest a role for the KDEL Receptor in promoting ENaC biogenesis.

## 68. Elucidation of the effectors of TNF $\alpha$ signaling that regulate liver repopulation

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Chronic liver disease is one of the leading causes of death worldwide. Currently, the only effective treatment for severe liver disease is transplantation. A major inhibitor of hepatocyte proliferation in diseased liver is inflammatory signaling through the Tumor Necrosis Factor alpha (TNF $\alpha$ ) cascade. However, TNF $\alpha$ -mediated activation of NF $\kappa$ B signaling can also exert anti-apoptotic effects. We previously performed a competitive overexpression screen of 43 genes thought to be involved in liver biology using the *Fah*<sup>-/-</sup> mouse, a model of toxic liver injury in which hepatocytes are damaged by the accumulation of toxic metabolites. In this model, hepatocytes rescued by ectopic expression of a functional copy of *Fah* can proliferate and repopulate the injured liver. In the screen, mice were injected with a pool of plasmids encoding the *Fah* gene and one of the 43 genes of interest. The plasmids encoding *Tnfr1*, the receptor for the cytokine TNF $\alpha$ , were the most depleted during liver repopulation. We hypothesized that activation of specific downstream effectors in the TNF $\alpha$  signaling cascade suppress hepatocyte proliferation, while other effectors promote proliferation. To test this hypothesis, we targeted the activation of 110 TNFR pathway genes using a CRISPR/Cas9 activation system in the *Fah*<sup>-/-</sup> rescue model. Our preliminary data suggest that activation of *Map3k8* transcription significantly promotes, and *Ripk3* inhibits, hepatocyte repopulation following injury. In future experiments, we will also test the effects of inhibition of TNFR pathway genes on liver repopulation following injury. Mapping of signaling downstream of TNFR1 within hepatocytes could identify targets for promotion of proliferation, which could serve as a potential alternative to transplantation.

## **69. An Optimized DNA encoded monoclonal antibody (DMAb) prevents Zika virus infection in mice and NHPs**

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Over 2 billion people globally are at risk of Zika virus (ZIKV) infection. Monoclonal antibody (mAb) clones isolated from human ZIKV patients have demonstrated protection in mouse and NHP models. Usage of MAbs for prevention of viral infections represents a new and important approach for intervening in outbreak situations. However, manufacturing, delivery, and distribution pose serious limitations and economic hurdles for field deployment. Thus, large at-risk global populations in developed and developing countries could benefit from additional approaches. We have reported a novel strategy for facilitated delivery of synthetic DNA-encoded mAbs (DMAbs) directly in vivo. This strategy utilizes CELLECTRA-EP® technology to deliver transient immunoglobulin (Ig) transgene to skeletal muscle for in vivo production and secretion. Using this approach, we engineered DMAbs expressing mAb ZK190, a highly potent, neutralizing Ab clone that binds uniquely to the ZIKV E protein. DMAb-ZK190 and variant DMAb-ZK190-LALA, designed to abrogate FcR binding, achieve peak levels of expression in vivo over 27.0 µg/mL and 62.1 µg/mL, respectively, with persistent IgG expression detected past 10 weeks. These levels provide 100% protection in a stringent lethal murine challenge model. Additionally, DMAb-treated mice display normal testes histology with no viral load, while untreated mouse testes are damaged by lingering effects of ZIKV. In NHPs expression levels of DMAb-ZK190 in sera reach over 1.0 µg/ml and DMAb-ZK190 protects NHPs from Zika infection. The role of the N-glycan profile of in vivo-produced DMAbs in challenge outcome is under investigation. Additional study of DMAb technology for rapid intervention in disease outbreak situations appears important for additional investigation.

## 70. Building a city-wide child trauma behavioral health system in Philadelphia naturalistic client outcomes

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The majority of youth experience a traumatic event by age 17 (Finkelhor *et al.*, 2009); thus, evidence-based trauma treatments like Trauma-Focused Cognitive Behavioral Therapy (TF-CBT) need to be available for the subset of youth who develop Post-Traumatic Stress Disorder (PTSD). In this study, we implemented TF-CBT in 15 clinics with 114 youth as part of a broader effort to implement trauma-informed care in Philadelphia. To evaluate the effectiveness of TF-CBT on youth symptoms in this naturalistic setting, we benchmarked our outcomes against efficacy and effectiveness studies. Youth averaged 12 years old ( $SD = 3.93$ ), were 56% female, and 52% African American. Using linear regression to estimate the effectiveness of TF-CBT from baseline to termination, we found statistically significant decreases in PTSD symptom severity ( $b = -4.45$ ,  $standard\ error\ [SE] = 1.96$ ,  $p = .02$ ,  $Cohen's\ D = .34$ ), and PTSD functional impairment ( $b = -.80$ ,  $SE = .33$ ,  $p = .02$ ,  $D = .38$ ). Although we found a statistically significant decrease in problem severity ( $b = -5.30$ ,  $SE = 2.55$ ,  $p = .04$ ,  $D = .29$ ), there was no change in general functioning ( $b = 2.58$ ,  $SE = 2.12$ ,  $p = .22$ ,  $D = -.14$ ). Per Reliable Change Indices (Jacobson and Truax, 1991), the percentages of youth with clinically significant improvements were 33% for PTSD symptom severity, 29% for PTSD functional impairment, and 48% for problem severity. The effect sizes for symptom improvement among the youth in our study were small compared to medium and large effect sizes among youth in the efficacy (Cohen *et al.*, 2004) and effectiveness (Jensen *et al.*, 2014) studies. We will conclude by discussing the implications of our results for future implementation trials of TF-CBT and other evidence-based practices.

## **71. Decreased phase amplitude coupling across laminar hippocampal CA1 following traumatic brain injury**

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Traumatic brain injury (TBI) is caused by mechanical insults to the head, often resulting in brain dysfunction including disruption of cognitive functions. We hypothesize that TBI may disrupt the timing between ensembles of neurons in hippocampal networks, and that this may underlie aspects of post-TBI cognitive dysfunction. Preliminary results demonstrated that TBI leads to a disruption of oscillatory organization in the hippocampus and dysfunctional entrainment to theta, potentially due to a compensatory response to loss of afferent input due to axonal injury. Cross-frequency coupling (CFC) and spike-local field potential (LFP) entrainment may support the organization of these neuronal ensembles, which can be affected by TBI. Using multichannel silicon probes, we simultaneously recorded laminar hippocampal field structure and CA1 neurons in behaving rats and pigs during an open field paradigm. Phase-amplitude coupling (PAC) was calculated for every possible pair of channels using the 1-20 Hz band as the phase of the lower frequency oscillation and 1-300 Hz band as the power of the coupled higher frequency oscillation. The entrainment of the neurons was calculated for each single neuron's firing properties, with every channel's LFP oscillations on the laminar probe for each frequency in 1-300 Hz band. Results show a reduction in PAC in injured rats relative to sham, predominantly between radiatum and pyramidal layers in theta-gamma bands, reflecting a loss of encoding synchrony between CA3-CA1, EC-CA1 and an interruption of sharp wave-ripple generation. These results will be also evaluated in pigs post rotational and focal injuries. These changes may lead to altered entrainment and the consequent disruption of neuron ensemble formation post injury.

## 72. Tunable microenvironment to enhance the delivery and response of stem cells for cartilage repair and regeneration

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Cartilage injuries represent one of the most common knee injuries, and often progress to joint-wide degeneration. Mesenchymal stem cell (MSC) injections have become a common treatment strategy. However, the localization of MSCs to the injury and their function upon arrival are not controlled. Therefore, we developed a tunable biomaterial designed to attract MSCs to damaged cartilage surfaces and to regulate their behavior to enhance repair.

**Methods:** Hyaluronic acid (HA) was methacrylated (~40% mod) and conjugated with fluorescent (FITC) and cell-adhesive (RGD) peptides. The material was oxidized to create aldehydes that form covalent links with exposed amines in damaged tissue. Biomaterial attachment to cartilage and micromechanics at the defect interface were quantified. The material was applied to bovine cartilage discs (6 mm diameter x 100  $\mu$ m thick) and cross-linked, and MSCs were seeded onto constructs for 24 hours. Cells were evaluated for attachment (F-actin) and mechanosensation (YAP/TAZ localization).

**Results:** The biomaterial was strongly attached to an ex vivo cartilage defect, and significantly improved mechanics at the damaged cartilage interface. The number of cells and cell spread area both increased with biomaterial application, indicating improved attachment. YAP/TAZ nuclear localization increased significantly with time of biomaterial cross-linking, confirming a mechanically tunable microenvironment that can potentially control cellular response.

**Discussion:** Our novel biomaterial microenvironment can be targeted to cartilage defects, recruit MSCs, and tune cellular response. Future work will optimize biomaterial attachment, cell adhesion and spreading, and microenvironment mechanics to promote desired stem cell differentiation.

### **73. Non-invasive optical cerebral blood flow and oxygenation monitoring during sudden cardiac arrest**

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Each year, there are approximately 300,000 cardiac arrests in the United States and nearly 90% of them are fatal due to brain injury (American Heart Association). Physicians guide their clinical care after resuscitation to prevent brain injury and patient death, however, the causes of patient deterioration are not well understood. Here we report multimodality measurements of cerebral hemodynamics using invasive brain monitors and a custom-built, non-invasive neurometabolic optical monitor (NNOM) during a patient's spontaneous cardiac arrest. The invasive monitors measured Intracranial Pressure (ICP) and microdialysis concentrations of brain metabolites through a cranial bolt in the patient's left frontal cortex. Standard clinical measurements were also collected: oxygen saturation from a pulse-ox on the patient's finger and the mean arterial pressure (MAP) from a radial artery. Cerebral Perfusion Pressure (CPP) can be calculated by subtracting ICP from the MAP. Our NNOM sensor measured cerebral blood flow (CBF) adjacent to the cranial bolt using a non-invasive plastic probe that sits on the patient's scalp. CBF is calculated using the Diffuse Correlation Spectroscopy (DCS) technique, which uses safe, near-infrared laser light. Only two other reports of similar measurements on humans have been published; we corroborate their respective findings with our novel technology and show deteriorating autoregulation after the cardiac arrest concomitant with poor outcome. This case report proves the feasibility and clinical utility of the optical monitoring technology (NNOM) in addition to potential physiological measures of a patient's decline in health.

## **74. Adenoviral proteins E1B55K and E4orf6 use non-degradative ubiquitination to regulate viral late protein expression**

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During early infection, viruses co-opt host signaling processes and counter host antiviral defenses in order to establish favorable conditions for late phase infection. Adenovirus is a DNA virus important both for its role in human diseases and for its archetypal insights into understanding virus-host interactions. Two adenoviral proteins, E1B55K and E4orf6, associate into a host ubiquitin ligase complex and redirect substrate recognition to promote an environment conducive to viral replication. Mutating these viral genes impairs production of late viral proteins and reduces viral progeny. Previous work identified E1B55K/E4orf6 substrates that are ubiquitinated and degraded upon infection, but these targets do not explain late viral protein deficiency. We identify new targets of the viral ubiquitin ligase that may account for the defects in the E1B55K-deficient virus. We expressed E1B55K and E4orf6, enriched for ubiquitinated proteins, and applied mass spectrometry to identify potential substrates. We combined these results with proteomic analysis to normalize ubiquitin levels against protein abundance. We show that ubiquitination occurs on proteins that decrease over time, characteristic of proteasomal degradation. We also detected ubiquitination on proteins unchanged in abundance, suggesting non-degradative signaling. Potential non-degradative targets are enriched in RNA-binding proteins, with hnRNPC and RALY among the most significantly modified. We show that these two proteins are specifically ubiquitinated by the E1B55K/E4orf6 complex and that they play a functional role in late viral protein production in an E1B55K-dependent context. Overall, we provide the first known example of viral-mediated non-degradative ubiquitin signaling to affect protein production.

## **75. Lovastatin causes a dose-dependent decrease of MCL1 in statin-sensitive ovarian cancer cell lines triggering cell death**

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Statins are drugs prescribed for hypercholesterolemia and have been intensely researched for decades. Some of this work has demonstrated, through experimental and epidemiologic approaches, that statins have anticancer properties. Evidence for the efficacy of statin therapy in cancer, however, is still controversial as many of the studies are retrospective. Although many reports show a trend toward a benefit of statin treatment, most never reach statistical significance.

Here, we demonstrate that certain ovarian cancer cell lines are exquisitely sensitive to treatment with statins. For example, three-day treatment with 10 $\mu$ M statin completely eliminates a confluent plate of OVCAR3 cells. Our ongoing studies reveal that ovarian cancer cells that are sensitive to statin treatment manifest an amplification of chromosome 1q21. Within this genomic region are the S100 proteins, previously identified as potential biomarkers in several cancer types, as well as the anti-apoptotic protein MCL1. We performed a Reverse Phase Protein Array (RPPA) analysis on high dose/short exposure and low dose/longer exposure statin treated cells. In both conditions, RPPA analyses clearly singled out a decrease in the protein level of MCL1 in statin-sensitive cell lines, while there was no such change in non-sensitive lines. After identifying MCL1 as a potential target, Western blot analysis demonstrated a dose-dependent decrease in MCL1 protein levels with increasing statin concentration. Given the anti-apoptotic role of MCL1, a statin-induced decrease in MCL1 protein level may push these statin-sensitive, and possibly MCL1-dependent, ovarian cancer cells to cross the apoptotic threshold, ultimately causing tumor cell death.

## 76. In vitro sensitivity to a novel poly(ADP-ribose) polymerase 1 (PARP-1) alpha-particle therapy agent in neuroblastoma

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**Introduction:** Neuroblastoma is the most common extracranial solid malignancy in childhood with only up to a 50% 5-year survival rate in high-risk cases. Compared to normal and other neoplastic cells, neuroblastoma exhibits overexpression of the nuclear enzyme PARP-1 which can be targeted for specific delivery of alpha-particles to DNA. One such therapeutic agent is [<sup>211</sup>At]MM4, a novel compound with an alpha-emitting radionuclide astatine-211 labeled to a small molecule PARP-1 inhibitor.

**Methods:** Five different neuroblastoma cell lines were each seeded on a 96-well-plate and treated with various concentrations of [<sup>211</sup>At]MM4, <sup>211</sup>At, KX1 (non-radioactive PARP-1 inhibitor analog), and <sup>211</sup>At+KX1 for 72 hours. ATP bioluminescence assay was used to quantify the survival fraction in each well. For each cell line and treatment condition, the effective concentration for 50% reduction in cell viability (EC<sub>50</sub>) was calculated using a sigmoidal dose response curve.

**Results:** The EC<sub>50</sub> for [<sup>211</sup>At]MM4 for the five cell lines, expressed in radioactivity, ranged from 620 pCi/mL to 97 nCi/mL. On a radioactive scale, [<sup>211</sup>At]MM4 caused cell death at concentrations 10<sup>2</sup>-10<sup>3</sup> times lower compared to unbound <sup>211</sup>At, as well as compared to a proportional mixture of free <sup>211</sup>At and KX1. On a molar scale, [<sup>211</sup>At]MM4 caused cell death at concentrations 10<sup>8</sup>-10<sup>9</sup> times lower than KX1, demonstrating no cell kill by biochemical inhibition of PARP-1 at therapeutic doses.

**Conclusion:** In neuroblastoma cell lines, delivery of an alpha-emitter to the cell nucleus by the astatinated PARP-1 inhibitor [<sup>211</sup>At]MM4 results in enhanced cytotoxicity without requiring enzymatic inhibition of PARP-1. This promising *in vitro* efficacy suggests [<sup>211</sup>At]MM has potential to be utilized *in vivo* and in other cancer types for broader application.

## **77. Oncogenic Notch promotes long-range regulatory interactions within hyperconnected 3D cliques**

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Chromatin loops enable transcription factor-bound distal enhancers to interact with their target promoters to regulate transcriptional programs. Although developmental transcription factors, such as active forms of Notch, can directly stimulate transcription by activating enhancers, the effect of their oncogenic subversion on the 3-dimensional (3D) organization of the cancer genome is largely unknown. By mapping chromatin looping genome-wide in Notch-dependent triple-negative breast cancer and B-cell lymphoma, we show that Notch regulates its direct target genes through establishing new long-range regulatory interactions, far beyond its well-characterized role as an activator of distal enhancers. Moreover, a large fraction of Notch-promoted regulatory loops forms highly interacting communities of enhancers and promoters, termed “3D cliques”. Loss- and gain-of-function experiments show that Notch preferentially targets hyperconnected 3D cliques that regulate the expression of crucial proto-oncogenes. Our observations suggest that oncogenic hijacking of developmental transcription factors can dysregulate transcription through widespread effects on the spatial organization of cancer genomes.

## **78. Downregulation of type I interferon responses in the tumor microenvironment triggers the stromagenic switch**

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Desmoplastic extracellular matrix (ECM) supports tumor growth and progression. This type of ECM is assembled by cancer-associated fibroblasts (CAFs), which are derived from resident fibroblasts and recruited mesenchymal cells that are pathologically activated into CAFs through a process called the stromagenic switch. The molecular mechanisms underlying this switch remain poorly understood. Here, we reveal a novel mechanism wherein downregulation of the type I interferon receptor (IFNAR1) in stromal cells of the tumor microenvironment (TME) induces the stromagenic switch. Our previous studies demonstrated that IFNAR1 undergoes ubiquitination-dependent degradation in the TME leading to the inactivation of type I Interferon signaling. Using a syngeneic mouse model of colorectal carcinoma, we found that robust tumor growth is associated with the accumulation of CAFs expressing FAP, and high levels of desmoplasia. Remarkably, tumors grew poorly in mice that express the ubiquitination-deficient stable mutant form of IFNAR1 (IFNAR1<sup>S526A</sup>) and these tumors exhibited reduced FAP expression and a diminished desmoplastic reaction. Bone marrow transplantation experiments demonstrated that the stromagenic switch, desmoplasia, and maximal tumor growth require the inactivation of IFNAR1 in non-immune tissues. Furthermore, co-injection of wild-type fibroblasts partially restored the ability of colorectal carcinoma cells to grow in the IFNAR1<sup>S526A</sup> knock-in mice. Altogether, our studies show that loss of IFNAR1 expression in stromal cells is a critical step in developing a tumor supportive microenvironment. Efforts to stabilize IFNAR1 in the TME could serve to prevent the stromagenic switch and ensuing desmoplasia and ultimately curtail tumor growth.

## 79. Heterochromatin dynamics during development restrict early cell differentiation

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Domains of transcriptionally repressed heterochromatin, marked by histone 3 lysine 9 trimethylation (H3K9me3), are reduced in embryonic stem cells compared to fully differentiated cells. However, the establishment and dynamics of closed regions of chromatin at protein coding genes, in natural embryologic development, has not been described. We developed a novel, antibody-independent method to isolate and map compacted heterochromatin from low cell number samples. Unexpectedly, we uncovered extensive, high levels of H3K9me3-marked, compacted heterochromatin at protein coding genes in early, uncommitted cells at the germ layer stage, undergoing profound rearrangements and reduction upon differentiation, concomitant with cell type-specific gene expression. Perturbation of the three H3K9me3-related methyltransferases revealed that H3K9me3 heterochromatin is required to maintain cell lineage fidelity. We propose a key role for chromatin-based restriction of gene activity via H3K9me3 heterochromatin during embryologic development.

## **80. Using dogs to help us develop an 'electronic nose' early detection system for ovarian cancer**

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Ovarian cancer is the seventh-most common form of cancer found in women, with a low five-year survival rate when found in later stages. However, when found in an early stage, ovarian cancer has a significantly higher survival rate. Presently, there is no early diagnosis system for ovarian cancer, resulting in later diagnoses and poorer prognoses of the disease. Dogs are able to recognize ovarian cancer, even in these earlier stages of the disease. We are training dogs to alert on malignant ovarian cancer when presented with plasma samples of donated from human patients. Dogs are presented with a 'scent wheel' full of different odors, including the target odor of plasma of ovarian cancer, as well as controls such as plasma of benign ovarian tumors and control plasma from individuals with no ovarian growths. When each dog is able to target the ovarian cancer with sufficient proficiency, we present them with a collection of the volatile organic compounds which constitute the ovarian cancer odor signature using analytical chemistry methods. This will allow the dogs to communicate to us what part of the ovarian cancer VOC profile smells like ovarian cancer. From here, we hope to make an 'electronic nose' that can analyze blood samples and serve as an early detection system for ovarian cancer.

## 81. Origin of the human gut virome in early life

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Samples from infant gut as early as one month after birth contain  $\sim 10^9$  virus like particles (VLPs) per gram of stool, but meconium samples (right after delivery) contain few or no VLPs, raising the question of how these populations originate. Deep virome sequencing revealed a median of 1 viral species in meconium samples compared to 20 and 23 in month 1 and 4 samples, respectively. Annotation of VLP sequence reads showed a median of 2% viral reads in meconium samples, but 58% and 68% in month 1 and 4 samples. These results suggest the viruses were not present in neonates in utero, but were acquired after delivery. We found that viruses that replicate on human cells were rare early in life (1 out of 20 infected by Herpesviridae in meconium and 2 out of 20 infected by Adenoviridae in month 1), though more prominent by 4 months of age (10 out of 20 infected by Adenoviridae, Anelloviridae, Caliciviridae or Picornaviridae). No endogenous retroviruses were observed in early gut virome. Replicating lytic phage were undetectable using *in vitro* phage plaque assay. VLPs appear to originate primarily as products of prophage induction, as shown for example by experiments in which isolated infant gut bacteria were induced to produce phage, VLP genomes sequenced, and VLP abundance in stool shown to correlate with the abundance of the host bacteria ( $P=0.008$ ). We thus propose that low level spontaneous induction accounts for the early life virome. For prophage, this represents a bet hedging strategy—prophages will potentially encounter myriad diverse environments outside the human body, and genome packaging in both bacteria and viral particles offer two chances for successful replication.

## 82. Mechanisms Regulating Articular Cartilage Growth and Morphogenesis

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Synovial joints are essential for skeletal function and body movement. Unfortunately, joints -chiefly articular cartilage (AC)- are highly susceptible to congenital- and age-related diseases and exhibit poor repair capacity that current clinical strategies fall short of amending. To improve these strategies, more information is needed on mechanisms of AC development. AC acquires its mature, multizone functional organization postnatally, and consists of: flat, lubricant-producing surface zone cells; round, column-aligned intermediate zone cells that resist loads; and mineralized, deep zone cells that attach to bone. Little is known about mechanisms that drive this specific organization. Recent work from our lab tackled these central issues using genetic lineage-tracing tools in mice. Data showed that while cell proliferation plays a minimal role, cell translocation and realignment may be major drivers of tissue morphogenesis. To explore mechanisms, we ask here if primary cilia regulate this process. These mechanical- and morphogen-sensing cell surface organelles are crucial in morphogenesis of many mammalian tissues; studies show their involvement in chondrocytes contributing to skeletal elongation, however their roles in AC were unknown. We used a conditional loss-of-function approach (*Ift88-flox*) targeting joint-lineage progenitor cells (*Gdf5-Cre*) and monitored structural and functional consequences on AC growth. My current findings show that mutant tissue exhibits a drastic drop in proteoglycan levels essential for tissue resilience, and lack of columnar organization of intermediate zone cells with negative consequences on overall tissue organization. Ongoing studies aim to investigate mechanical and morphogenetic mechanisms accounting for these phenotypic effects.

### **83. Capturing the single-cell origins of skin cancer by live imaging**

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A key unanswered question in the cancer field is how the same oncogenic mutation causes a single cell to form tumors whereas most cells with the same mutations have no noticeable effect on homeostasis. Studies addressing it have been limited by a lack of in vivo models that enable the long-term tracking of individual cell fate after acquisition of tumor-driver mutations. A major hurdle is the inability to genetically manipulate and visualize stem cells at the single cell level in their native environment within intact live organs. To overcome it, I use live intravital microscopy of the adult mouse skin to study the first steps of tumor initiation in the skin.

In the skin, homeostatic balance is maintained by highly regulated stem cells that replenish cells lost through terminal differentiation. Spontaneous gene mutations can cause stem cells to deviate from homeostasis, and clonally and uncontrollably expand to ultimately develop into skin tumors. I will recapitulate this process in vivo by using a combination of inducible genetic systems to alter the expression of genes frequently mutated in skin carcinoma in single clones belonging to specific stem cell populations. Genetically altered and normal stem cells are differentially labeled with fluorescent reporters to track the clonal growth dynamics of both populations in the intact skin by live imaging. Altogether, this analysis will allow me to dissect the complex cell- and non-cell autonomous regulatory mechanisms of mutant stem cell contributions to early tumorigenesis.

#### **84. Kit inhibition reduces type I interferon signaling in the tumor microenvironment of gastrointestinal stromal tumors**

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Type I interferon (IFN) signaling is vital to tumoral MHC class I (MHC I) expression and facilitates the anti-tumoral lymphocyte response. Gastrointestinal stromal tumor (GIST) is a sarcoma caused by an activating *Kit* mutation and is sensitive to imatinib (IM), a specific Kit inhibitor. In patients, IM-treated GIST have lower MHC I expression, potentially limiting the efficacy of immunotherapy.

**Rationale:** IFN signaling regulates MHC I genes as part of the anti-viral response. Based on RNAseq data from *Kit*<sup>V558Δ/+</sup> mice, a murine model that produces an intestinal GIST, both IFN and MHC I related genes were decreased with IM, suggesting Kit inhibition may reduce tumoral IFN signaling and MHC I expression.

**Methods:** *Kit*<sup>V558Δ/+</sup> mice were treated with IM to study changes in MHC I expression and IFN signaling by flow cytometry and western blot. Blocking anti-IFNAR antibody was given for 3 weeks to abrogate IFN signaling. Human GIST cell lines were treated with IFNα with or without IM to study their direct effects.

**Results:** MHC I expression on tumor cells was reduced by 50% in *Kit*<sup>V558Δ/+</sup> mice treated with IM by MFI (n=5/group). Similarly, mRNA expression of *IFNb1* in bulk *Kit*<sup>V558Δ/+</sup> tumor after IM was diminished by 8-fold. To show that MHC I reduction was directly due to IFN, we blocked IFN signaling in *Kit*<sup>V558Δ/+</sup> mice and found MHC I decreased by 25% by MFI, along with decreased p-Stat1 signaling by western. In vitro, human GIST cells stimulated with IFNα showed increased MHC I and pStat1 by western at 24h. The addition of IM reduced MHC I expression 15% by MFI and 50% by mRNA.

**Conclusion:** IM reduced MHC I in GIST by decreasing IFN production and signaling via Stat1. Therapies that reverse this may have additive effect and potentiate immunotherapy for GIST.

## **85. The utility of pupillary light reflex as an objective biomarker of acute concussion in the adolescent athlete**

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**Background/Rationale:** Visual deficits and autonomic dysfunction have been well recognized following concussion. Testing of the pupillary light reflex (PLR) is a simple, non-invasive, and objective approach to examine the autonomic nervous system. The aim of this study was to objectively evaluate adolescent pupillary responses to a light stimulus after a concussion and compare them to baseline responses in adolescents.

**Methods:** In this prospective cohort study, PLR was assessed in 93 (45 female, 48 male), currently non-concussed adolescent athletes (ages 14-18) during their pre-season. Seven athletes (ages 14-17) sustained a concussion and had post-injury assessments of PLR longitudinally through their recovery. PLR was obtained in response to a brief white light stimulus using a hand-held pupillometer. During each assessment, three monocular trials were performed in each eye alternatively, with subsequent averaged responses for each eye.

**Results:** Six out of the seven concussed athletes showed response enhancement, defined as increased responsivity of the pupil relative to baseline. Enhancement was noted by a steady state diameter increase of 24% (median 18%), minimum pupil diameter increase of 17% (median 11%), and a maximum constriction velocity increase of 28% (median 33%) following concussion (mean initial day of evaluation = 6 days post injury), which then decreased during the recovery process to pre-injury or below initial pre-injury baseline measurements.

**Conclusion:** Pupil responsivity was found to be significantly enhanced after concussion compared to baseline measurements which waned over time during recovery. Assessment of dynamic PLR responses has potential utility as an objective biomarker to aid in concussion diagnosis.

## 86. mTORC1 activation in lung mesenchymal progenitors leads to defective alveolarization and age-dependent lung degeneration

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Mammalian target of rapamycin (mTOR) controls cell growth and metabolism in health and disease, and therefore, represents an attractive therapeutic target. mTOR pathway plays a crucial role in the fetal organ development, as well it is an important conserved mediator of the aging. mTOR signaling is activated in a vast array of diseases, many of which are linked to aging, including major diseases of the lung, such as idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and rare lung disease lymphangioleiomyomatosis (LAM). Tumor suppressor tuberous sclerosis complex 2 (TSC2) is known to negatively regulate mTORC1. To determine cell-type specific effects of mTORC1 upregulation on lung homeostasis, we generated *Tsc2* deletion in the mouse lung mesenchymal progenitors using a lung-specific *Tbx4-Cre*. As a result, we observed mTORC1 activation in several populations of the adult mouse lung mesenchyme, including PDGFR $\alpha$ <sup>+</sup>, PDGFR $\beta$ <sup>+</sup>, PECAM<sup>+</sup>, and  $\alpha$ SMA<sup>+</sup> cells.

Mesenchymal loss of *Tsc2* produced alveolar enlargement phenotype arising at birth, followed by age-dependent progressive emphysematous lung degeneration and vasculature remodeling. In agreement with the observed phenotype, *Tbx4/Tsc2* KO lungs demonstrated increased compliance and decreased elastance on a pulmonary function test. One-year old mice developed areas of lung fibrosis accompanied by inflammation. Importantly, treatment with mTORC1 inhibitor Rapamycin slowed down the progression of alveolar enlargement. Collectively, our study demonstrates that: (1) *Tsc2* loss in mesenchymal progenitors progressively impairs lung alveolarization, induces vasculature remodeling, and fibrosis, and (2) *Tsc2* gene expression is essential for normal lung development and homeostasis.

## 87. Upregulation of MHC class II in intestinal epithelial cells from patients with VEO-IBD

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Very Early Onset Inflammatory Bowel Disease (VEO-IBD) is an autoimmune disease that results in inflammation and damage in the intestine. The contribution of epithelial cell-autonomous effects to VEO-IBD pathogenesis is not clear. We performed RNA sequencing on colonoids (3D primary cell cultures derived from colon biopsies) obtained from patients with VEO-IBD (diagnosed <6), pediatric IBD (onset of symptoms between 6 and 18) and control patients without IBD. We found that the antigen presentation pathway was significantly upregulated in VEO-IBD compared both to controls and pediatric IBD. Interestingly, mRNAs that were upregulated in the antigen presentation pathway are involved in MHC class II, not MHC class I. Increased MHC class II gene expression was verified by qPCR in additional patients. Furthermore, VEO-IBD colonoids treated with IFN $\gamma$  upregulated MHC class II more robustly than colonoids from control patients. Taken together, these data suggest that an increase in MHC class II signaling in intestinal epithelial cells could be a mechanism by which intestinal inflammation is propagated in patients with VEO-IBD. Future studies will determine the relative contribution of increased MHC class II genes to epithelial cell survival, proliferation, and barrier function.

## **88. X-chromosome inactivation in human B cell and fidelity of X-silencing during SLE**

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X chromosome has high density of immunity related genes which may contribute to the sex bias in autoimmunity as observed in lupus. To balance dosage of X linked genes between male and female, one of the X chromosome is randomly inactivated in female cells. Previous studies have shown that some immunity related genes are expressed from the inactive X chromosome (Xi) in lymphocytes, and their expression level correlates with lupus severity. In the present study, we investigated Xi maintenance and expression of X-linked genes in B cell subsets from lupus patients and healthy individuals. We found that human naïve and memory B cells lack the canonical XIST RNA localization to the Xi. However, in-vitro stimulation triggers return of both XIST RNA and heterochromatic marks (e. g. H3K27me3, H2A-Ubiquitin and MacroH2A1) with concomitant loss of euchromatic mark (e. g. H3K4me3) from the Xi. Strikingly, we did not observe re-localization of XIST RNA back to Xi upon in-vitro stimulation of naïve and memory B cells derived from pediatric lupus patients. Consistent with this observation, chip-seq analysis revealed overall higher-level occupancy of active chromatin marks (H3K4me3 and H3K4me2) with ATAC-seq revealing overall more open chromatin regions on X chromosome (both active and inactive alleles combined) of lupus B cells compared to that of healthy individuals. In addition, RNA-seq analysis revealed overexpression of many X-linked immunity related genes in lupus B cells compared to that in healthy individuals. Our findings suggest that dysregulated Xi maintenance in lupus B cells may cause reactivation of autoimmunity related genes from Xi leading to sex bias in lupus.

## **89. Impact of skin microbiome on wound healing in diabetic foot ulcers**

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Diabetes is a group of metabolic diseases associated with several long-term complications. Risk of developing diabetic foot disease and ulceration is alarmingly high at a 15-20 % lifetime incident. The micro-environment of a diabetic foot ulcer (DFU) differs from other types of wounds (e.g. increased inflammatory cytokine production, prolonged PMN infiltration, hypoxia) and is also believed to result in a delayed healing phenotype. Microbes respond to external cues that influence their physiology, such as changes in temperature, pH, and host immune effectors. A detailed understanding of the mechanisms by which microbial responses in a DFU occur at both the individual and community level is not clearly defined. We have previously shown that microbiome community instability was associated with faster healing and improved outcomes in patients with DFU. In the current study we have investigated how alteration of host-genes by microbiota alters wound healing responses in primary keratinocytes and skin via ex vivo analysis. This work aims to shed light on host-microbe interactions involved in altering wound healing characteristics in chronic wounds.

## 90. Suppression of IL-1b-mediated inflammation by HPV

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High-risk human papillomavirus (HR-HPV) causes 5% of cancers worldwide, including anogenital and oropharyngeal cancers. Most HPV infections are asymptomatic and resolve spontaneously. Persistent infections can progress to precancerous lesions. The life cycle of HPV strictly follows the differentiation process of the stratified squamous epithelium. HPV infects basal keratinocytes, which are often exposed to the environment through abrasion. Upon infection, the expression of viral genes promotes cell cycle progression of the host cell. As the infected keratinocytes progress through their natural differentiation process, HPV replicates and packages its genome into virions. The virus is spread when the terminally differentiated cells shed due to natural processes or abrasion. HPV infections often do not elicit a robust immune response, which has been attributed to the lack of lytic viral shedding and evasion of host immunity. Previous works by others have found that HPV-transformed cells or precancerous lesions have lower mRNA level of the pro-inflammatory cytokine interleukin-1b (IL-1 $\beta$ ), and single nucleotide polymorphisms in gene of the inflammasome pathway, including *IL1B*, are associated to worse outcome of HPV infections. HR-HPVs have been reported to degrade IL-1 $\beta$  through the proteasome pathway; however, the mechanism of reduced *IL1B* transcript remains unknown. The role of IL-1 $\beta$  in HPV infections and subsequent tumorigenesis is also elusive. The goal of our work is to understand the importance of *IL1B* in HPV pathogenesis. Our preliminary data shows that expression of HR-HPV E7 in keratinocytes leads to a reduction of *IL1B* mRNA, and we are currently determining how E7 deregulate *IL1B* and its effects on the HPV life cycle and if this phenotype is specific to HR-HPV.

## 91. Comparison of Methods to Address Short Stature-Related Artifacts on Childhood Bone Density

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Childhood is a critical period of bone growth, with greater than 90% of adult bone mass being accrued by age 20 y. Numerous pediatric chronic conditions affect longitudinal growth and bone accretion, potentially increasing fracture and osteoporosis risk in adulthood. Due to the close link between stature and bone density measures by DXA in children, areal bone mineral density (aBMD) values can be difficult to interpret in those with low height-for-age Z-scores (HAZ). Current pediatric recommendations suggest using bone mineral apparent density (BMAD) or HAZ-adjusted aBMD (aBMDHAZ) in children with short stature, but it is unknown whether these measures account for height-related artifacts throughout childhood. We compared bone density measures across short (HAZ<-1), average (HAZ=-1 to 1), and tall (HAZ>1) children ages 5-9.9 y, 10-14.9 y, and ≥15 y to assess their performance in accounting for stature. An unbiased measure will minimize the correlation with HAZ. Data were from the longitudinal Bone Mineral Density in Childhood Study, including healthy children ages 5-19 y at baseline (N=2014, 46% male, 22% African American), with up to 6 annual measures. In all age groups, aBMD-Z was lower in short vs. tall children (all p<0.0001). In those <15 y, differences in BMAD-Z between short and tall children were modest (both p<0.02), but in those ≥15 y, the difference was minimal (p=0.05). In those <15 y, aBMDHAZ-Z did not differ between short and tall children. However, aBMDHAZ-Z was considerably greater in short vs. tall children ≥15 y (p=0.0003). Whereas both BMAD and aBMDHAZ reduce stature-related artifacts on bone density, neither perform consistently throughout childhood. Further work is needed to refine these methods, and in doing so, will help improve clinical care.

### Funding

This work was supported by the National Institute of Child Health and Human Development (NO1-HD-1-3228, -3329, -3330, -3331, -3332, and -3333), the General Clinical Research Center (5-MO1-RR-000240), the CHOP Clinical and Translational Research Center (UL1-RR024134), the National Center for Advancing Translational Sciences (TL1TR001880), and the CHOP Research Institute.

## 92. Role of Akt signaling in regulation of skeletal muscle physiology

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**Background:** Skeletal muscle (SkM) accounts for more than 40% of the body mass, providing means for locomotion at the expense of cellular energy. SkM relies on distinct metabolic pathways to meet this energetic demand. Insulin and insulin-like-growth factor 1 signaling control SkM growth and function and defects in these underlie a host of SkM diseases; however, the molecular mechanisms mediating these effects remain ill-defined.

**Rationale:** To understand the molecular mechanisms controlling insulin and IGF-1 regulation of SkM physiology, we generated mice lacking Akt, an essential downstream signaling molecule with SkM specific deletion of Akt2 (M-Akt2KO), the predominant Akt isoform in SkM, and Akt1 and Akt2 (M-AktDKO), to delete both the Akt isoforms.

**Methods:** Muscle function was assessed using multiple assays including fiber typing, *ex vivo* performance and treadmill running.

**Results:** Despite a reduction of ~90% of total Akt following Akt2 deletion alone, M-Akt2KO had normal muscle mass and no apparent phenotype. In contrast, M-AktDKO mice have significant reduction in muscle mass resulting in lower absolute force generation and increased fatigability in EDL (glycolytic) and Soleus (oxidative) muscle and impaired performance accompanied by the loss of oxidative fibers. Mechanistically, the reduction in muscle mass in M-AktDKO mice can be rescued *in vivo* by combined activation and inhibition of the mTORC1 and Foxo1 pathways.

**In conclusion,** Akt signaling is critical to maintain the oxidative properties of SKM *in vivo* and the complete loss of SkM specific Akt signaling leads to reduced muscle mass resulting in profound abnormalities in SkM physiology. Importantly, activation of mTORC1 and inhibition of Foxo1 are both required and sufficient to control muscle mass *in vivo*.

### **93. Oral delivery of enhanced IGF-1 expressed in antibiotic-free lettuce chloroplasts to treat muscle/bone disorders**

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Human insulin-like growth factor 1 (IGF-1) plays important roles in development and regeneration of skeletal muscle and bones. Current clinical IGF-1 requires daily injections but the lack of e-peptide or glycosylated form decreases functional efficacy. Growth hormones used in dental medicine enhance bone regeneration but requires flap debridement surgical delivery, decreasing patient compliance. This study addresses several such key protein drug production and delivery concerns. Pro-IGF-1 with e-peptide (fused to cell penetrating peptides) was codon optimized and expressed in lettuce chloroplasts. The antibiotic resistance (*aadA*) gene was quickly excised via homologous recombination of direct repeats and marker free chloroplast genomes were maintained in lettuce plants in the next generation, with high level expression of Pro-IGF-1. Pro-IGF-1 was stable and maintained proper folding in lyophilized plant cells when stored at ambient temperature for several months/years. Chloroplast derived CTB-Pro-IGF-1 stimulated growth of cultured human oral keratinocytes, gingiva-derived mesenchymal stromal cells and mouse osteoblasts in a dose-dependent manner, facilitating various dental/medical applications. Mice orally gavaged with lyophilized plant cells expressing CTB-Pro-IGF-1 or PTD-Pro-IGF-1 significantly increased IGF-1 in sera and muscle in male and female mice with equal efficiency. Biopharmaceutical expression in plant cells free of the antibiotic resistance gene, with long-term ability to store lyophilized cells at ambient temperature and the convenience of repetitive oral delivery for long-term treatment should enhance affordability and patient compliance by eliminating daily injections or surgical implantations.

## **94. Implantable three-dimensional scaffolds for potential neuromuscular tissue engineering applications**

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**Introduction:** Neuromuscular damage can result from physical trauma like volumetric muscle loss, spinal cord and peripheral nerve injury. Ideally, surgical intervention in cases with neuromuscular damage/muscle loss would entail fabrication and implantation of bioengineered nerve-muscle complexes, however such has yet to be developed. The present study describes biofabrication of innervated tissue engineered skeletal muscle through neuromuscular co-culture on a polymeric scaffold.

**Materials and Methods:** The polymeric scaffolds were fabricated using concentrated solution of gelatin and were crosslinked using a natural crosslinker to provide stability. Differentiated skeletal myocytes and spinal motor neurons were subsequently engineered to form separate three-dimensional (3D) aggregates. Both the cell types were plated on the polymeric scaffolds and cultured in co-culture media. At terminal time points, the cells were fixed and immunostained for axonal marker, skeletal muscle actin and neuromuscular junctions.

**Results and Discussion:** The crosslinked scaffolds were observed to be stable over time in comparison to non-crosslinked ones. Skeletal myocytes grown on crosslinked gelatin scaffolds exhibited robust proliferation and differentiated to form long, matured myofibers. Motor neuron aggregates co-cultured with differentiated myofibers showed dense axonal outgrowth, innervation into myofibers and formation of neuromuscular junctions.

**Conclusions:** The polymeric scaffolds supported nerve-muscle co-culture thereby forming 3D neuromuscular constructs which can be used in the short term as in vitro test beds towards exploring neuromuscular disorders. In the long term, these constructs could be used as an implant to replace/repair volumetric muscle loss in patients.

## **95. Deletion of expanded CGG repeats lowers Fmr1 mRNA and increases FMRP levels in Fmr1 knock-in mice**

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The trinucleotide repeat in the 5' untranslated region of the Fragile X Mental Retardation 1 (FMR1) gene is normally 5-44 CGG repeats in length. Premutation (55-200 CGG repeats) alleles of this triplet repeat can result in Fragile X-associated Tremor/Ataxia Syndrome (FXTAS), a late onset neurodegenerative disorder. Whereas a full mutation (greater than 200 CGG repeats) is the predominant cause of the neurodevelopmental disorder Fragile X Syndrome (FXS). This study evaluates the use of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 to delete the expanded CGG repeat for therapeutic benefit. We tested multiple gRNA sequences for cutting efficiency in HEK 293 cells prior to packaging two gRNAs and Cas9 into two AAV1 vectors.

Fmr1 knock-in mice harboring a premutation sized allele upstream of Fmr1 were injected in the striatum with the optimized AAV1-CRISPR constructs and three weeks post injection the mice were euthanized. The striatum was isolated and DNA, RNA or protein was evaluated. PCR amplification of genomic DNA followed by sequencing showed partial CGG repeat deletion along with 3-48 nucleotides upstream and downstream of the repeats. Sequencing revealed an intact transcriptional start site and start codon. In contrast to control-treated mice, where Fmr1 transcripts are elevated approximately 3-fold, AAV1-CRISPR treated mice had Fmr1 mRNA levels similar to WT levels. The FMR1 protein, FMRP, which is reduced in the Fmr1 knock-in mice by approximately 50-75% reverted back to WT levels in mice injected with the CRISPR construct. These results are the first in vivo report of editing the Fmr1 trinucleotide repeat with CRISPR and shows rescue of abnormal mRNA and protein expression.

## 96. Sub-cellular acyl-CoA analysis reveals the nucleus as a distinct metabolic compartment

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Acyl-CoAs are major metabolic intermediates and key substrates for post-translational modification of proteins. Cellular acyl-CoA abundance correlates with protein acylation. Notably, acetyl-CoA abundance is coupled to histone acetylation and gene expression. Thus, acyl-CoAs connect metabolic state to cell signaling. However, acyl-CoAs have distinct roles in different sub-cellular compartments, and rigorous methods to specifically interrogate acyl-CoA metabolism in mitochondria, cytosol, or nucleus have been lacking. We developed Stable Isotope Labeling of Essential Nutrients in Cell culture - Sub-cellular Fractionation (SILEC-SF) as a method to resolve sub-cellular acyl-CoA distribution. SILEC-SF uses rigorous internal standard controls during sub-cellular fractionation and processing for accurate metabolite quantitation by mass spectrometry. We validated our approach by examining metabolic adaptation to hypoxia, a key feature of tumor development, which involves metabolic rewiring with well-described compartmentalized features. By tracing <sup>13</sup>C-labeled substrate incorporation into acyl-CoAs over time, we observed kinetically distinct acyl-CoA pools in the mitochondria, cytosol and nucleus. Incorporation of <sup>13</sup>C-acetate into acetyl-CoA was slower in the nucleus versus the cytosol, consistent with the inefficient use of exogenous acetate for histone acetylation (nuclear) versus lipid synthesis (cytosolic). This work demonstrates that the nucleus is subject to distinct metabolic regulation, which specifically impacts epigenetic modification. Thus we present a method for examining compartmentalized metabolism to understand the role of metabolites in cell signaling processes.

## 97. Changes in low-frequency fluctuations in patients with obsessive-compulsive personality disorder

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**Background:** Obsessive-compulsive personality disorder (OCPD) is one of the most prevalent personality disorders which is characterized by a pervasive pattern of preoccupation with orderliness, perfectionism, and mental and interpersonal control, at the expense of flexibility, openness, and efficiency. Its neural basis is unknown. The aim of this study is to investigate the alterations in resting state brain activities in OCPD patients compared to matched healthy controls to fill this gap.

**Methods:** Spontaneous neural activity were examined in 37 OCPD patients and 37 healthy controls (HC) by analyzing the amplitude of low-frequency fluctuation (ALFF) in the resting state.

**Results:** Compared with HC, OCPD had increased ALFF in medial frontal gyrus, precuneus, caudate, and insula, and decreased ALFF in lingual gyrus.

**Conclusions:** Alterations in ALFF in these specific brain regions suggest that OCPD patients may be associated with abnormal activities in the Default Mode Network, visual network and reward system.

## **98. Erlotinib improves photodynamic therapy response in a 3D model of malignant pleural mesothelioma**

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We have found that lung sparing surgery with intraoperative photodynamic therapy (PDT) produces remarkably extended survival for patients with malignant pleural mesothelioma (MPM). Nevertheless, most patients treated with this approach go on to experience local recurrence as a component of treatment failure, so it is essential to determine mechanisms of tumor resistance to these therapies and develop methods to improve tumor response. We have previously shown that benzoporphyrin-mediated PDT transiently activates EGFR/STAT3 and induces EGFR nuclear translocation in ovarian and lung cancer, and inhibiting EGFR with erlotinib can increase the PDT sensitivity of these cells. Additionally, we have seen that higher EGFR expression associates with worse outcomes in patients receiving Photofrin-mediated PDT for MPM, and the extensive desmoplastic reaction associated with MPM influences tumor phenotype and response to therapies. Since extracellular matrix (ECM) proteins accrued during stroma development can alter EGF signaling within tumors, we have characterized 3D MPM models to determine their response to Photofrin-mediated PDT after pretreatment with erlotinib. Our MPM cell lines formed a range of acinar phenotypes when grown on ECM gels that recapitulate the locally invasive phenotype of MPM in pleura and endothoracic fascia. Using these models, we found that EGFR inhibition dramatically increases the direct cytotoxicity of PDT through a mechanism that involves increased apoptotic cell death. Taken together with emerging evidence that EGFR inhibition may improve survival of lung cancer patients through both immunologic as well as direct cell killing mechanisms, these results suggest that erlotinib enhanced PDT may significantly improve outcomes in patients with MPM.

## **99. Differential spontaneous brain activity changes in basic and higher-order brain networks after sleep deprivation**

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Sleep plays a critical role in maintaining the health, whereas sleep deprivation (SD) has a broad range of adverse effects on human performance and neural functioning. Previous neuroimaging studies have demonstrated the detrimental effects of SD on regional brain function. However, it remains unclear whether SD affects different brain resting-state networks in a same or different manner. In this study, we used resting-state functional magnetic resonance imaging to investigate the effects of SD on spontaneous brain activity in different brain resting-state networks measured by the amplitude of low-frequency fluctuation (ALFF). Thirty-eight healthy volunteers were scanned both following the 9 hour time-on-bed baseline sleep and after one night of total SD. Compared to the day following baseline sleep, SD significantly increased ALFF in the thalamus, motor network, visual network, and auditory network, but decreased ALFF in the Default Mode Network (DMN), salience network, attention network, and executive network. These findings suggest that SD has differential effects on spontaneous brain activity in basic brain networks and higher-order brain networks.

## 100. The non-canonical NF- $\kappa$ B pathway regulates marginal zone cellularity

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The pro-inflammatory transcription factor NF- $\kappa$ B plays key roles in regulating immune and inflammatory diseases, and is activated through distinct mechanisms named the canonical and non-canonical (NC) pathways. Activation of NF- $\kappa$ B in vascular endothelial cells (EC) promotes secretion of inflammatory cytokines and expression of adhesion molecules that contribute to the initiation and maintenance of inflammatory responses. Although the significance of canonical NF- $\kappa$ B signaling in EC has been extensively studied, the role of NC NF- $\kappa$ B in these cells is not well understood.

Previous findings from our lab have shown that the NC NF- $\kappa$ B pathway is activated upon Lymphotoxin (LT) $\alpha$  Receptor ligation within ECs. To determine the physiological role of this pathway, we developed mouse models targeting IKK $\alpha$  in ECs by crossing IKK $\alpha$ <sup>ff</sup> mice with Tie2-cre or Cdh5-cre animals. In both these models, the mice lack lymph nodes (LNs) and display impaired peripheral B cell maturation. As hematopoietic progenitors are partially derived from Tie2+Cdh5<sup>+</sup> hemogenic endothelial cells, we found that both the EC and haematopoietic compartments lack IKK $\alpha$  in these mice and therefore together contributes to this phenotype. Despite lacking all peripheral lymph nodes, the spleens were overtly intact in both the IKK $\alpha$ <sup>Tie2</sup> and IKK $\alpha$ <sup>Cdh5</sup> mice. Our experiments using fluorescence microscopy have revealed reduction in the cellularity within the Marginal Zone B cell compartment with significant loss of metalophilic and marginal zone macrophage subsets that are bordering these regions. Together, these findings indicate that the NC NF- $\kappa$ B pathway is critical for immune homeostasis within the splenic white pulp.

## **101. Changes in respiratory tract and gut bacteria attributable to antibiotic exposure during long-term acute care**

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### Objectives

Long-term antibiotic exposure increases the risk of acquiring antibiotic-resistant bacterial infections. We sought to quantify the in vivo response of specific bacteria to antibiotics and predict the onset of drug resistance in those bacteria. For this, we drew upon a cohort of long-term acute care hospital (LTACH) patients from whom dense longitudinal oral, endotracheal aspirate, and stool specimens were collected.

### Methods

Longitudinal specimens of each type were selected from 27 subjects in the LTACH cohort for whom complete antibiotic administration data were available. Bacterial composition was assessed by amplifying and sequencing the 16S V1-V2 region, resolving variants with DADA2 (v1.6.0), and assigning taxonomy with the Silva database (v132). Bayesian model fitting and analysis was performed using R (v3.5.0) and Stan (2.1.7).

### Results

We evaluated a total of 472 subject-days of study enrollment across the 27 subjects (median 15 days/subject). For each specimen type and bacterial taxon, we fit a Bayesian generalized linear model of read counts parameterized with the previous subject-day's abundance and antibiotic exposure, along with partially-pooled subject- and specimen-level intercepts. The effect of antibiotic exposure on bacteria varied widely by individual subject. For example, the estimated proportional change of endotracheal *Staphylococcus aureus* after intravenous vancomycin exposure ranged from -0.48% to 0.05% (median: -0.01%). Other bacteria and antibiotics showed similarly wide ranges.

### Conclusions

Hierarchical, autoregressive models provide an efficient way to analyze longitudinal studies. Individual partially-pooled slopes for each subject's response to antibiotic exposure allow us to characterize both the overall effect and to identify subjects with unusual responses. In future work, we will incorporate interactions between multiple antibiotics and correlate subject-level antibiotic effects with clinical outcomes.

## **102. Differences between the matrix and cell phenotype of the neonatal and adult extrahepatic bile duct: implications for injury**

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Biliary atresia (BA) is the leading indication for pediatric liver transplants. It is characterized by obstruction of the extrahepatic bile duct (EHBD), leading to disruption of bile flow, inflammation and ultimately liver fibrosis and cirrhosis. The cause of BA is still unknown although environmental factors have been implicated (e.g. viral infections and toxins). Retrospective studies show that injury occurs prenatally as elevated levels of conjugated bilirubin are detected within days of birth. Mothers, however, do not develop the disease, suggesting neonatal susceptibility. Understanding differences between the neonatal and adult EHBDs is therefore key to understanding the pathogenesis of BA. EHBDs were isolated from adult BALB/C and Collagen  $\alpha 1(I)$ -GFP mice and pups at postnatal days 0-15. These were imaged using transmission electron microscopy, second harmonic generation microscopy and stained for matrix components and cell type markers. The submucosal space of EHBDs in adult mice consists of collagen I/III bundles, proteoglycans, hyaluronic acid and elastin rich ECM interspaced with fluid-filled spaces. The collagen bundles are also lined by fibroblasts. Conversely, the neonatal EHBDs only has very limited elastin and collagen fibrils suggesting these will have poor response to mechanical stress (e.g. obstruction). Additionally, neonatal submucosal cells, unlike the majority of adult cells, are actively producing collagen I, contain large amounts of rough endoplasmic reticulum and express the myofibroblast marker  $\alpha$ SMA suggesting that these metabolically active neonatal cells are primed for fibrosis. In conclusion, here we identify two factors which confer neonatal susceptibility to BA as well as identify the previously uncharacterized fibrotic cell of the EHBD.