SEQUENCE MONITORING IN THE FRONTAL CORTEX
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Performing sequential tasks such as making your breakfast are an integral part of daily life. The majority of previous studies have focused on motor sequences or non-sequential abstract control, rather than these kinds of more abstract sequential tasks. To address this gap, we asked human participants to repeatedly perform simple four-item sequences of shape and color judgements during fMRI scanning. We found a novel dynamic in the rostrolateral prefrontal cortex (RLPFC) where activation ramped up through the four items each sequence and reset at the beginning of each new sequence. TMS to RLPFC during the same task selectively produced an increasing pattern of errors as each sequence progressed, mirroring the fMRI activation. Effects in the RLPFC during fMRI and two independent TMS experiments dissociated from two other prefrontal control regions. These results show that RLPFC is necessary for sequential control and resolution of uncertainty during sequence performance. Current work focuses on dissociating some of the processes that underlie sequential task control: task execution, sequential monitoring, and sequence memory. Preliminary results from a sequence monitoring task suggest that the dynamics of sequential control may follow a similar hierarchical, rostral to caudal, more to less abstract gradient as has been found in non-sequential, hierarchical control tasks.

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MICRORNA-146A IS NECESSARY AND SUFFICIENT TO MAINTAIN CARTILAGE HOMEOSTASIS
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Osteoarthritis (OA) is the most prevalent aging-related joint disease and a major cause of disability worldwide. Many studies have suggested possible involvement of miR-146a in physiological and pathological processes of articular chondrocytes. However, its role in cartilage tissue maintenance is largely uncharacterized. To determine the role of miR-146a in osteoarthritis
in vivo, we systemically characterized the mice in which miR-146a is either deficient in whole body or over-expressed specifically in chondrogenic cells. MiR-146a deficient mice develop early-onset of aging associated OA by 6 months of age. Conversely, miR-146a chondrogenic over-expressing mice are resistant to aging associated OA with reducing ADAMTS5 levels. To understand the underlying mechanism by which miR-146a regulates OA pathogenesis, we further demonstrated that Notch1 is a direct target of miR-146a. Co-transfection of ATDC5 cells with miR-146a mimic significantly reduced luciferase activity in cells transfected with wild type Notch1 3'-UTR but not with deletion or mutated seeding site. Moreover, immunohistochemistry results showed the expression of Notch1 was up-regulated in miR-146a knockout and down-regulated in miR-146a chondrogenic overexpressing mice. Therefore, we provided the first genetic evidence that deficiency of miR-146a led to the early onset and progression of OA through regulating Notch signaling. Our study suggests that deficiency of miR-146a may lead to OA and that stimulating chondrogenic expression of miR-146a by either inducing its expression or providing it exogenously may serve as a potential therapeutic for OA to inhibit cartilage degeneration.

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Eposterboard #3

ANTI-INFLAMMATORY EFFECTS OF INTER-ALPHA INHIBITOR PROTEINS (IAIPS) AFTER HYPOXIC-ISCHEMIC (HI) BRAIN INJURY IN NEONATAL RATS

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Problem: Hypoxic Ischemic Encephalopathy (HIE) is the leading cause of disabilities in newborns. IAIPs are immunomodulatory serine protease inhibitors that we have shown can reduce pathological brain injury after HI, but the mechanism(s) by which IAIPs exert their neuroprotective effects remains unknown. Increases in microglial proliferation, neutrophil infiltration and release of matrix-metalloproetinase-9 (MMP-9) represent important components of CNS neuroinflammation after HI. The objective of this study is to test how IAIPs affect cellular mediators of HI-related neuroinflammation. Methods: We used the Vannucci model to induce HI brain injury in P7 rats assigned to non-ischemic sham-control (Sham, n=18), right carotid ligation plus hypoxia placebo-treated (Isch-PL, n=20), and right carotid ligation and hypoxia IAIP-treated (Isch-IAIP, n=19) groups. IAIP (30 mg/kg) or PL was given intraperitoneally at 0, 24 and 48 h after HI. 72 h after HI, brains were prepared for analysis. Sections were stained immunohistochemically with Iba-1 (microglia marker), or myeloperoxidase (neutrophil marker) and MMP9 antibodies. Quantification was performed by stereological analyses in specific brain
regions without knowledge of group assignment. Results: Exogenous IAIPs reduced the number of microglial cells in the hippocampus (P<0.01) neutrophils in the parenchyma of the damaged hemisphere (P<0.01) and corpus callosum (CC, P<0.05) in male but not female rats. Sections treated with both MPO and MMP9 markers exhibited co-localization and IAIPs reduced the number of MMP9 positive neutrophils in the cortex (P<0.05) of male rats. Conclusions: IAIPs appear to exert neuroprotective effects by reducing ischemia-related markers of inflammation in select brain regions of male neonatal rats.

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Eposterboard #4

NEW FEATURES AND APPLICATIONS OF DATABASE FOR PREECLAMPSIA V2.0

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Problem: Preeclampsia is a complex disease. It is a multi-system hypertensive disorder of pregnancy. Methods: We built a Database for Preeclampsia (dbPEC) including curated articles, clinical features, genes, and preeclampsia phenotypes and gene networks. It is publicly available (http://ptbdb.cs.brown.edu/dbpec) We present 1) New user interface and functionality for curators site of dbPEC: User interface has simple navigation features, accelerates curation process and makes curation experience smoother. Curators are now able to record additional preeclampsia information about Race/Ethnicity, detailed tissue sources. 2) One of the important practices of dbPEC is the gene sets. They can be customized according to phenotype, and concurrent conditions of the disease. The extracted gene sets can be used as 'filters' for gene set enrichment analysis, candidate gene analysis or other analyses of publically available. In this demonstration we sought to identify enrichment of these gene sets in well phenotyped, published placental genome-wide expression data sets. Results: One expression array compared severe early onset preeclamptic patients (n=5) to normotensive patients (n=4) and we found significant enrichment for the gene set of fetal, early onset genes (p<.0001). For a second expression array comparing severe preeclamptic patients (n=8) to normotensive patients (n=8) both the fetal, early onset gene set and the fetal, severe preeclamptic gene sets were nominally enriched (p<.05). Conclusions: We used knowledge-based inferences by examining the combinatorial effect of preeclampsia-associated genes. Since it leverages the genetic architecture of preeclampsia, it is more successful than univariate analysis of genome-wide expression data.
DIETARY SUPPLEMENTATION WITH MEDIUM-CHAIN TRIGLYCERIDES REDUCES CANDIDA GASTROINTESTINAL COLONIZATION IN PRETERM INFANTS
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Problem: Candida spp. are an important cause of sepsis in premature infants. GI colonization with Candida is a common site of entry for disseminated disease. The objective of this study was to determine whether a dietary supplement of medium-chain triglycerides (MCT) reduces Candida colonization in preterm infants. Methods: Preterm infants with Candida colonization receiving enteral feedings of either infant formula (n=5) or breastmilk (n=7) were randomized to MCT supplementation (n=8) or no supplementation (n=4). Daily stool samples were collected to determine fungal burden during a 3 week study period. Infants in the MCT group received supplementation during 1 week of the study period. The primary outcome was effect on fungal burden during the supplementation period as compared to the time periods before and after supplementation. Results: GI Candida fungal burden was similar in control and treatment groups before supplementation (p=0.81). In the treatment group, there was a significant reduction in GI fungal burden during the supplementation period as compared to the period before supplementation (p<.0001). This reduction persisted after stopping supplementation (p<.0001). Fungal burden in the control group remained consistent. Conclusion: Dietary supplementation with MCT may be an effective method to reduce Candida colonization in preterm infants.

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IMPROVING THE TIMING OF CYTOKINE DELIVERIES IN WOUND HEALING VIA MAGNETICALLY RESPONSIVE HYDROGELS
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Problem: In Chronic wounds, although inflammation initiates repair and helps clear infections, prolonged inflammation can sabotage the normal process of wound healing and damage the wound site. The delivery of anti-inflammatory cytokines like interleukin 4 (IL-4) and interleukin 10 (IL-10) can convert this inflammatory response to a more prohealing response by transforming the phenotype of macrophages from pro-inflammatory (M1) to anti-inflammatory (M2). This suggests a potential drug delivery strategy if these cytokines can be delivered in a delayed manner, after inflammation has done its job (representing an unknown duration of time). The objective of this study is to design a biomaterial system that can initially deliver interferon gamma (IFN-γ)—a proinflammatory factor—followed by delayed IL-4 and/or IL-10 delivery in response to magnetic stimuli.

Methods: Our biomaterial consists of two compartments: a porous gelatin outer compartment designed to recruit macrophages and promote an initial pro-inflammatory (M1) phenotype, and a biphasic, iron-oxide-laden porous alginate inner compartment which was designed to deliver IL-4 and/or IL-10 when magnetically deformed to shift the response to anti-inflammatory. Cytokine delivery profiles were quantified using ELISA.

Conclusions: We have demonstrated this system’s ability to initially release a proinflammatory cytokine (IFN-γ), while retaining the anti-inflammatory cytokines (IL-4 and IL-10) for several days. The timing and rate of IL-4 and IL-10 deliveries could be controlled by applying magnetic fields at different times and at different frequencies. We believe that application of this material will help regulate the inflammatory response in wound healing applications.

Endothelium-specific NADPH Oxidase-derived ROS Improves Cardiac Function in a Post-Myocardial Infarction (MI) Model.
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Background: Ischemic heart disease (IHD) is one of the leading causes of death in the United States. Reactive oxygen species (ROS) are generally observed to be increased during many cardiovascular diseases including IHD. Recent results in our lab of increasing NADPH oxidase
(NOX)-derived ROS improving vascular endothelial function have contradicted previous notion that decreased ROS improves vascular function. One of the most effective ways to study IHD has been to mimic human myocardial infarction in an animal model. This is achieved by occluding the Left Anterior Descending Artery (LAD) in mice. Using this model, we study how the heart can be protected from damage resulting from IHD. In the current study, we look at supraphysiological (~2-fold) increase in endothelial NOX-ROS in an MI model.

**Hypothesis:** We hypothesized that an increase in NOX-ROS in endothelial cells (EC) would improve cardiac function after MI, and we would see an increase in vascular density and reduction in infarct area.

**Method:** Our binary (Tet-ON/OFF) conditional transgenic mouse (Tet-Nox2:VE-Cad-tTA, hence termed Tg-Nox2) induces 1.8±0.42-fold increase in ROS in EC. DCF fluorescence assay and Mitosox assay were performed to validate the increase in ROS on mouse heart ECs (MHEC). Tet On and Tet Off Tg-Nox2 mice that were exposed to eight weeks of increased EC-ROS were subject to LAD ligation. After four weeks of surgery, an echocardiogram was performed to gain understanding on the cardiac function. Co-immunofluorescence staining on heart sections were used to quantify vascular density, EdU staining for angiogenesis, and trichrome staining was used to quantify infarct size. Using Western blots and aspartate assays, we analyzed CPT1a expression levels and corresponding aspartate synthesis activity as measure for precursor of dNTP synthesis.

**Results:** DCF fluorescence assay and Mitosox assay show an increase in ROS in Tet-OFF Tg-Nox2 mice. NOX-ROS improved recovery of post-MI heart by increasing angiogenic activity in the ischemic regions as shown by co-immunostaining using anti-CD31 and anti-SMA antibodies, and EdU immunohistochemistry. We demonstrate that there was an increase in capillary density (by 38±6.45; p<0.05) and angiogenesis (by 2-fold). Tg-Nox2 animals showed reduction in infarct size by Trichrome staining and improvement in cardiac function including EF, LVEDV and LVESV by echocardiography. Western blot analysis showed an increase in CPT1a in Tet-OFF Tg-Nox2 MHEC compared to Tet-ON mice. A corresponding increase in Aspartate synthesis suggested an increase in the synthesis of dNTP precursor (aspartate) in Tet-OFF Tg-Nox2 ECs compared to Tet-ON MHEC.

**Conclusion:** These findings suggest that an increase in EC-ROS may play an important role in cardiovascular health and myocardial preservation.

**Eposterboard #8**

Macrophase Dicer Expression is Required for VEGF-A-induced Arteriogenesis

During ischemia, inflammatory arteriogenesis is determined by macrophage VEGF-A expression. A number of microRNA’s have been identified to downregulate VEGF-A and VEGF-mediated angiogenic processes. We sought to determine the microRNA(s) that were most critical to regulating macrophage VEGF-A expression by genetically deleting Dicer in myeloid cells. In a hindlimb ischemia model of angiogenesis and arteriogenesis, we found reduced rescue of
hindlimb perfusion in macrophage Dicer −/− animals. Surprisingly, this was associated with decreased macrophage VEGF-A production and leaky, dysfunctional arteries as evidenced by microCT angiograms, indicating that Dicer expression and consequent microRNA biogenesis are required for appropriate VEGF-A expression and VEGF-mediated arteriogenesis. The mechanism of reduced macrophage VEGF expression involved decreased VEGF-A mRNA stability with decreased association of VEGF-A mRNA with the RNA-stabilizing protein HuR. Our results support a paradigm shift in the perception of Dicer expression and microRNA biogenesis as a negative regulator of VEGF-A expression and implicate microRNA biogenesis in promoting macrophage VEGF-directed arteriogenesis in response to ischemia. Identification of this novel mechanism of VEGF-A regulation has strong implications in the development of targeted therapeutic strategies that can promote appropriate arteriogenesis in the setting of obstructive vascular disease or inhibit inappropriate arteriogenesis in the setting of malignant oncological diseases.

Eposterboard #9

The Effect of Equilibrative Nucleoside Transporter 1 on Pseudomonas Aeruginosa-Induced Acute Lung Injury

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Acute lung injury (ALI) is characterized by lung edema and inflammation and results in 40% mortality. Pseudomonas aeruginosa infections, due to multidrug resistance, often causes ALI particularly in patients with compromised immunity. Adenosine, a signaling nucleoside increased upon tissue injury, has been shown to protect against ALI through activation of adenosine receptors (ARs). Extracellular and intracellular adenosine concentrations are regulated by equilibrative nucleoside transporters (ENTs). Whether altering adenosine signaling and uptake can prevent or treat bacterial P. aeruginosa-induced ALI is unknown. We hypothesize that increased extracellular adenosine by inhibiting ENT1 will protect against P. aeruginosa-induced ALI via activation of ARs. ENT1 pharmacological inhibitors and null mice as well as agonists and antagonists directed against adenosine receptors A2AR and A2BR were used to test this hypothesis. We found that P. aeruginosa infection induces ALI in C57BL/6 mice in a dose dependent manner.
Pharmacological inhibition of ENT1 by NBTI significantly attenuates *P. aeruginosa*-induced ALI, as assessed by wet-to-dry lung weight, bronchoalveolar lavage (BAL) protein levels, BAL inflammatory cells, and pulmonary function, which was evaluated by static lung compliance, tissue damping, and tissue elastance. NBTI also increased bacterial clearance in mice infected with *P. aeruginosa*. Additionally, NBTI attenuated *P. aeruginosa*-induced IL-1β levels in the BAL of mice. In consistent with ENT1 inhibitor, ENT1 null mice had attenuated ALI, as assessed by BAL protein and BAL cell counts, in mice infected by *P. aeruginosa*. Finally, agonists of A2AR and A2BR mimic NBTI blunting *P. aeruginosa*-induced ALI, whereas antagonists against either A2AR or A2BR diminished the protective effect of NBTI against *P. aeruginosa*-induced ALI. These results suggest that blocking NBTI protects against *P. aeruginosa*-induced ALI via adenosine-mediated activation of both A2AR and A2BR. Inhibition of ENT1 may be a novel approach to prevent and treat *P. aeruginosa*-induced ALI.

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**Eposterboard #10**

PATIENT-DERIVED MELANOMA VARIANTS OF DNA POLYMERASE THETA EXHIBIT ALTERED POLYMERASE ACTIVITY

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**Problem:** DNA is randomly damaged on a daily basis by many factors including environmental exposure or random replication errors. This damaged DNA can lead to genomic instability and eventually cancer if left unrepaird. DNA polymerases play an important role in maintaining the genome during DNA repair, but poorly functioning DNA polymerases can contribute to genetic instability by incorporating the wrong nucleic acid, introducing mutations. DNA polymerase Theta (Pol θ) or POLQ performs low fidelity repair for certain types of DNA damage, is upregulated in several types of cancer, and associated with poor survival rates. There are limited research studies exploring the link of a dysfunctional Pol θ mutant to cancer. We have identified several POLQ mutations from patient-derived melanomas and hypothesize that these variants will have altered polymerase activity compared to wild-type Pol θ that will result in increased rates of mutagenesis. Methods: Pol θ variants L2538R and E2406K located in the palm domain and fingers domain of Pol θ were generated via site-directed mutagenesis. Variants were expressed in *E.coli* and purified via affinity chromatography. Highly purified protein was assayed for qualitative activity including polymerization of correct and incorrect nucleotides. Results: Preliminary studies suggest that the variants demonstrate a propensity to misincorporate nucleotides resulting in altered base-selection activity compared to wild-type Pol θ. Conclusion: Our data suggests these melanoma derived Pol θ mutations repair DNA differently compared to wild-type Pol θ, which may contribute to overall genomic instability.
DECREASING LUNG TISSUE TIE1 COINCIDENT WITH INCREASING PLASMA TIE1 EXPRESSION IS ASSOCIATED WITH SURVIVAL IN MURINE MODEL OF INDIRECT ACUTE LUNG INJURY.

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Following severe trauma, many patients develop life-threatening sequelae. Hemorrhage (Hem), often a component of trauma, has been identified as predisposing for vascular dysfunction and subsequent organ failure. To better understand the mechanism(s), we have focused on the development of acute respiratory distress syndrome (ARDS) following Hem and subsequent septic challenge (CLP) in a murine model. In this model we show that the Angiopoietin (Ang)/Tie2 pathway plays a significant role in mediating endothelial cell (EC) homeostasis and activation, as well as dysfunction. Ang-1 & Ang-2 mediate EC activation through competitive binding to Tie2 expressed on ECs. Ang-1 binding induces Tie2 phosphorylation (pTie1) and signaling for downstream anti-inflammatory and anti-apoptotic proteins and vessel barrier integrity. Alternatively, Ang-2/Tie2 binding promotes increased neutrophil interactions and vascular permeability. We have demonstrated that EC interaction with Hem-primed neutrophils contributes to EC activation and Ang-2 release, and that depletion of neutrophils prior to Hem/CLP decreases Ang-2 and increases pTie2. Recent publications, as well as our preliminary data, suggest that the EC orphan receptor, Tie1, may also play a role in modulating EC activation by forming a complex with Tie2. In the experiments presented here, we show that lung tissue Tie1 steadily increases 24-60 hours following Hem/CLP. In mice that survive past 60 hours (the time point were we see greatest mortality in our model) we observe a decrease in lung tissue Tie1 expression, increase in pTie2 and an increase in Tie1 protein in the blood. These findings suggest that the shedding of Tie1 from ECs may indicate the resolution of EC activation.

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Title: Composition of mutant Swi/Snf complexes
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Abstract:
The 12 subunit Swi/Snf chromatin remodeling complex is conserved from yeast to humans. It functions to alter nucleosome positions by either sliding nucleosomes on DNA or evicting histones. Interestingly, 20% of all human cancers carry mutations in subunits of the Swi/Snf complex. Many of these mutations cause protein instability and loss, resulting in partial Swi/Snf complexes. Though several studies have shown that histone acetylation and activator dependent recruitment of Swi/Snf regulate its function, it is less well understood how subunits regulate stability and function of the complex. Using functional proteomic and genomic approaches we have assembled the network architecture of yeast Swi/Snf. In addition, we find that subunits of the Swi/Snf complex regulate occupancy of the catalytic subunit Snf2, thereby modulating gene transcription. Our findings have direct bearing on how cancer-causing mutations in orthologous sub-units of human Swi/Snf which show similar organization for human Swi/Snf complex. Additionally, we have identified unique interactors of yeast Swi/Snf and show that these interactors can affect regulation of the complex.

Eposterboard #13

Eposterboard #14

Poster title: Role of bacterially regulated vitamin A metabolism in immune homeostasis in the gut

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Poster abstract:

Intestinal epithelial cells (IECs) are the first barrier of defense to a 100 trillion bacteria in the gut. Hence, their function needs to be tightly regulated, so as to avoid invasion by commensals, and at the same time respond to pathogens. One way they maintain barrier integrity is by secreting antimicrobial peptides (AMPs). AMPs production by IECs can occur by cytokines such as interleukin-22 (IL-22). IL-22 can be secreted by immune cells such as T cells and innate lymphoid cells (ILCs). IL-22 has been shown to play a crucial role in protection from bacterial infection by aiding in AMPs production and helping in the regeneration of the epithelium.

Retinoic acid (RA), a metabolite of Vitamin A, is a key regulator of immune function in the gut. It has been shown that RA can regulate function of tissue resident lymphoid cells including the enhancement of IL-22 production. Currently, there is no real knowledge on the source of RA needed for IL-22 regulation. While studies state that RA is produced predominantly by dendritic cells (DCs), it is important to highlight that IECs are the first cells to take up Vitamin A, and they have the requisite machinery to synthesize RA. Our recent finding is that the lack of IEC intrinsic RA synthesis is able to affect IL-22 producing cells. Even more interesting, the gene retinol
dehydrogenase 7 (rdh7), involved in RA synthesis, is bacterially regulated. Altogether, these findings emphasize the importance of understanding epithelial cell intrinsic vitamin A metabolism regulated by bacteria.

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**Eposterboard #15**

Structure, assembly, and aggregation of RNA-binding proteins in Inclusion Body Myositis and ALS
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Inclusion body myositis (IBM), the most common muscle disease associated with aging, has recently been linked to the formation of protein aggregates, but the mechanism of toxicity is currently unknown. We have used NMR spectroscopy combined with in vitro microscopy to complete an atomic resolution characterization of aggregation-prone “prion-like” domains of RNA-binding proteins genetically and histologically linked to IBM. We find that the missense mutation at a conserved position in two RNA-binding proteins, heterogeneous nuclear ribonucleoprotein (hnRNP) A2B1 and hnRNPA1, that causes hereditary forms of IBM alters the structure of the monomeric protein to a more aggregation-prone conformation. Furthermore, we find that hnRNP A2 low complexity domain and full-length forms reversible self-assembled structures in vitro that resemble RNA granules where hnRNPs physiologically localize. Like RNA granules, these protein dense structures are reversible and appear to be liquid-like. Combining in vitro fluorescence recovery after photobleaching (FRAP) microscopy with direct atomic-resolution characterization of these assemblies, we find that the prion-like domains within these in vitro granules remain structurally disordered even upon assembly. We also map the sites of hnRNP A2 interaction with the binding partner cyclophilin A. These results demonstrate that characterization of the RNA granule birthplaces of protein aggregates in IBM, ALS, and frontotemporal dementia is within reach.

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IDENTIFICATION AND CHARACTERIZATION OF A NOVEL ANTIGEN PFCDPK-5 FOR PEDIATRIC MALARIA VACCINE


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Malaria is among the leading causes of mortality in children under five years of age worldwide, with most of these deaths resulting from Plasmodium falciparum infection. Despite decades of research, no vaccine candidate has been shown to confer significant protection to children. The immediate goals of this study are to gain an immunological understanding of anti-PfCDPK-5 antibodies in preventing parasite maturation and egress. In ongoing antigen discovery studies, we pioneered a high-throughput differential whole proteome screening method to identify targets of antibodies that protect children from severe malaria and identified Schizont Egress Antigen-1 (Raj et al. Science 2014). In a parallel screening experiment, we identify PfCDPK-5 as a potential vaccine candidate. The localization study and growth inhibition activity were evaluated as per our published methods. We identified antibodies against PfCDPK-5 protein only in malaria resistant children's sera, not in susceptible kids sera. The preliminary data shows that PfCDPK-5 phosphorylates PfSEA-1 another important protein responsible for egress of parasites. The confocal microscopy demonstrates the localization of the protein on merozoite of the rupturing schizont. The polyclonal antibody generated by recombinant DNA vaccine shows significant growth inhibition activity in vitro assays. Our preliminary data demonstrate that immunization with PbCDPK-5 can generate robust antibody response and protect the mice from parasitemia and greatly extend the life of the mice after lethal P. Berghei Anka malaria parasite challenge. In the present study, we validate a rationally identified vaccine candidate; PfCDPK-5 using integrated translational approaches that harness high-throughput molecular techniques and in vitro functional assays.

Soft, Conductive Hydrogels as an Interface between Tissue and Electrode

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Neuroprosthetic intervention are strategies aimed for treating a wide range of disorders, long term neuroprosthetic treatments require a robust connection between hard metallic electrodes and soft tissue that must sustain their electrical connection with tissues for prolonged periods of time. Whether these electrodes are implanted or placed on skin, they required a soft, conductive interface to minimize the results of implantation and mechanical mismatch, and remain hydrated to provide connection between electrode and tissue. And to open the door toward wearable electronics. Our aim here is to develop soft, conducting hydrogel-based electrode materials and characterize their sustained mechanical and electrical properties. These gels were made by entrapping a conductive polymer (poly (3-4, ethylenedioxythiophene) (PEDOT)) within different hydrogel network (we studied poly (acrylic acid (PAA) and alginate to make hydrogels) and different conditions (room temperature vs subfreezing temperature) to obtain different microstructure. These gels exhibit wide range of mechanical properties; stiffness (0.5-21 kPa for PAA and 1.3-15.2 kPa for alginate), strain of failure (50 % - > 90 % for PAA and 70 % - 83 % for alginate) and toughness (1.4-1.6 kJ/m³ for PAA and 2.5-23.3 kJ/m³). On the other hand, these gels, regardless of their composition, have conductivity of about 1 S/cm. This conductivity was much higher than neural tissues, making them well-suited for stimulating and sensing in neuroprosthetic applications. Independence of conductivity from composition provides the opportunity of selecting gels based on properties demonstrated by application.

Eposterboard #18

Magnetically Responsive Hydrogels for Optimizing Osteo-differentiation by Delaying BMP-2 Delivery
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Problem: Bone diseases and injuries affect millions annually and often include expensive treatments. While traditional bone grafts can provide the framework for bone regeneration, they suffer from major drawbacks including donor site morbidity. Tissue engineering approaches have produced biomaterial bone grafts that are additionally capable of directing bone regeneration through the presentation of growth factors, but do not mimic the temporally complex nature by which bone growth is controlled. We hypothesized that delaying the delivery of BMP-2 (bone morphogenetic protein-2) can enhance bone progenitor osteo-differentiation. The objectives of this study were to investigate the effect of delaying BMP-2 delivery on osteo-differentiation and to develop a biomaterial system capable of delaying the delivery of BMP-2.

Methods: D1 MSCs were seeded sparsely at 1500 cells/well and exposed to osteo-supplemented medium with or without BMP-2. Osteocalcin secretion was measured over 2 weeks using ELISA. In order to magnetically control the delivery of BMP-2, ferrogels were made by casting 7 wt% Fe₃O₄ with 1 wt% Alginate and crosslinking with 2.5 mM adipic acid dihydrazide (AAD) in the presence of a magnet. Gels were frozen at -20 °C and lyoohilized to create porous, magnetically deformable biphasic structures. Results: When BMP-2 is delivered
to mMSCs before they reach dense population, osteo-differentiation was inhibited. Delayed BMP-2 delivery enhanced mMSCs osteo-differentiation compared to early BMP-2 delivery. We also demonstrated that this delayed delivery can be achieved using our biphasic ferrogels. We expect such a system to be highly valuable in optimizing deliveries in bone regeneration research.

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Eposterboard #19

PREECLAMPSIA IS A SYNDROME OF PROTEIN MISFOLDING AND AGGREGATION

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Problem: We have demonstrated that protein aggregation is associated with the pathogenesis of preeclampsia. However, how aggregated proteins accumulate in the placenta and cause pathology is poorly understood. Whether these protein aggregates (PAs) can be detected in the serum or urine of preeclampsia patients prior to the onset of disease still remains to be elucidated.

Methods: Sera and placental tissue from preeclamptic/normal pregnancy women were evaluated for the presence of protein aggregates using a modified filter retardation assay or an ELISA-based assay and dual staining in combination with ProteoStat dye that uniquely binds to PAs. Functional alterations in the unfolded protein response (UPR) and autophagy-lysosome degradation pathway (LDP) were analyzed in a cellular model of ER stress. Results: We found a large amount of placental transthyretin (TTR) and Aβ staining co-localized with ProteoStat dye in the PE placenta. Importantly, our data show higher levels of TTR aggregates in sera from preeclamptic women as early as 12-14 weeks. Mechanistically, persistent treatment with ER stressors, low oxygen tension or inflammatory cytokines, impaired UPR and LDP and led to aggregation of both TTR and Aβ in trophoblast cells. Conclusions: Persistent ER stress impairs the UPR and LDP, leading to deposition of PAs in the placenta and their release into maternal circulation. Placental injury from PAs may contribute to the onset of PE. Risk for PE may be predicted through detection of PAs in sera from women early during pregnancy. (Supported by COBRE for Perinatal Biology; Brown DEANS Award, Connie Howes Women’s Research Award, and Oh-Zopfi Award)

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ELUCIDATION OF TRANSCRIPTIONAL NETWORKS THAT PRESERVE ADULT NEURAL STEM CELLS

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Adult neural stem cells (NSCs) are the source of new neurons in the adult mammalian brain, and are a promising source of regenerative therapies. The brain contains both actively dividing stem cells and very slowly dividing NSCs, termed quiescent NSCs. These quiescent NSCs are the source of proliferative NSCs, are actively maintained, and have the potential to be reactivated and form new neurons in response to external stimuli. While the quiescent NSCs are ultimately the critical reserve population of NSCs, the precise mechanisms by which these cells are maintained and how they are activated remain unknown. Our goal is to determine how quiescent NSCs are directly regulated at the transcriptional level. Our published and preliminary data implicate the longevity-associated transcription factor FOXO3 as a key regulator of quiescent stem cells in the adult. FOXO3 is a central regulator of both aging and stem cells, and mice lacking FOXO3 have an age-related depletion of NSCs. Using ChIP-seq, we identified neural stem cell-specific FOXO3 targets, and found that they include important regulators of cellular homeostasis during aging, including proteostasis and metabolic targets. Using functional assays overexpressing or ablating FOXO factors in NSCs, we found that FOXO3 is a direct regulator of autophagy in these cell, indicating that FOXO3-mediated cellular quality control is a key mechanism for maintaining a healthy pool of NSCs in the adult brain. Together, these experiments provide new insight into how the conserved pro-longevity FOXO transcription factors function in NSCs to regulate cellular homeostasis and longevity.

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GENE AND NETWORK ANALYSIS OF COMMON VARIANTS REVEALS NOVEL ASSOCIATIONS IN MULTIPLE COMPLEX DISEASES

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Problem: Genome-wide association studies (GWAS) typically lack power to detect genotypes significantly associated with complex diseases, where different causal mutations of small effect may be present across cases. A common, tractable approach for identifying genomic elements
associated with complex traits is to evaluate combinations of variants in known pathways or
gene sets with shared biological function. Such gene-set analyses require the computation of
gene-level \( p \)-values or gene scores; these scores are also useful when generating hypotheses
for experimental validation. However, commonly used methods for generating gene scores are
computationally inefficient, biased by gene length, imprecise, or have low true positive rate
(TPR) at low false positive rates (FPR), leading to erroneous hypotheses for functional
validation. Methods: Here we introduce a new method, PEGASUS, for analytically calculating
gene scores. Then, we use gene scores from PEGASUS as input to HotNet2 to identify
networks of interacting genes associated with multiple complex diseases and traits. Results:
PEGASUS produces gene scores with as much as 10 orders of magnitude higher numerical
precision than competing methods. In simulation, PEGASUS outperforms existing methods,
achieving up to 30% higher TPR when the FPR is fixed at 1%. Using PEGASUS scores as input
to HotNet2, we discover networks that include genes previously associated with acute
lymphoblastic leukemia, ulcerative colitis and waist–hip ratio, as well as novel candidate genes
for these phenotypes. In contrast, existing methods fail to identify these networks. We also
identify networks for attention-deficit/hyperactivity disorder, in which GWAS have yet to identify
any significant SNPs.

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Eposterboard #22

STIMULI-RESPONSIVE HYDROGELS FOR DIRECTING SPROUTING AND MATURATION
OF VASCULATURE
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Problem: Growth of mature vascular networks is needed in myriad biomedical applications and
requires initial delivery of pro-angiogenic factors (to initiate sprouting) followed by pro-
maturation factors (facilitate network maturation). Here, we demonstrate a hydrogel system
capable of initially releasing a pro-angiogenic factor (Vascular Endothelial Growth Factor,
VEGF) followed by delayed delivery of a pro-maturation factor (Platelet Derived Growth Factor,
PDGF). Methods: A two-compartment hydrogel system was fabricated to sequentially release
VEGF and PDGF. The outer compartment was a macroporous gelatin. The inner compartment
was made by casting 7 wt\% Fe3O4 in 1 wt\% alginate with 2.5 mM AAD crosslinker in the
presence of a magnet. Gels were freeze-dried to yield porous, biphasic magnetically deformable
structures. Gels were loaded with 1-0.5 \( \mu \)g of VEGF and 250 ng of PDGF in the outer and inner
compartment, respectively. Release vs. time was quantified using ELISA. Results: Our
hydrogel system was able to rapidly release VEGF proportional to the initial loading. PDGF
release was controlled with magnetically stimulated release rates all being statistically greater
than non-stimulated gels. Conclusions: We successfully developed a hydrogel system that can
deliver VEGF at earlier time points followed by delayed release of PDGF after external stimulation. Timing and rate of PDGF delivery were remotely controlled by time, duration and frequency of applied magnetic field. Because it is unknown what timing of PDGF delivery might be beneficial in different therapeutic scenarios, the ability to magnetically control the timing of PDGF delivery in real-time is expected to have high clinical value.

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Eposterboard #23

CHI3L1-CRTH2 AXIS IS A BIOMARKER AND THERAPEUTIC TARGET IN PULMONARY FIBROSIS

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Rationale: Tissue injury and exaggerated fibroproliferative repair are cardinal features of pulmonary fibrosis (PF) including Idiopathic Pulmonary Fibrosis (IPF). The mechanisms that drive the tissue injury and fibrotic repair responses in these disorders are poorly understood, and therapies that impact the course of PF are very limited.

Hypothesis: We hypothesize that CHI3L1, a prototypic chitinase like protein, and its receptors are dysregulated in and play critical roles in the generation of lung fibrosis. We also hypothesize that these responses are largely mediated by its receptor CRTH2 and that an index of the ability of CHI3L1 to induce M2 macrophage differentiation is a biomarker for CHI3L1 and or CRTH2 sensitivity in IPF.

Methods: We characterized the dysregulation and role(s) of CHI3L1 and its receptors, specifically CRTH2, in lung-relevant murine models of pulmonary fibrosis. We also compared the ability of CHI3L1 to stimulate M2 differentiation in circulating monocytes from IPF patients and healthy controls and determined the role that CRTH2 plays in these responses.

Results: In the preliminary studies, we demonstrate that CHI3L1 utilizes CRTH2 to optimally promote tissue fibrotic responses. We also demonstrate that optimal CHI3L1-induced M2 differentiation is mediated via a CRTH2-dependent mechanism. They indicate that the sensitivity of blood monocytes to CHI3L1 induced M2 differentiation may serve as a biomarker that predicts responsiveness to CHI3L1 or CRTH2 based interventions.

Conclusions: This project will add insight as to the utility of CHI3L1-CRTH2 as therapeutic targets and a biomarker that predicts responsiveness to CHI3L1 or CRTH2 based interventions in pulmonary fibrosis.

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**Eposterboard #24**

**SIRNA DELIVERED BY NANOPIECE FOR TREATMENT OF POST-TRAUMATIC OSTEOARTHRITIS**

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When joints sustain injuries, stimulation of inflammatory mediators can cause degeneration of the articular cartilage and the subsequent development of post-traumatic osteoarthritis (PTOA). Currently, there are no clinically approved therapies to prevent acute PTOA. SiRNA provides great therapeutic potential to specifically inhibit disease gene expression during arthritis progression. However, it is extreme challenging to deliver negatively charged RNAs into avascular, dense and negatively charged cartilage matrix. We developed an innovative nano-delivery vehicle, named “Nanopiece (NP)”, whose unique nano-rod shape and tiny size enable it to effectively infiltrate cartilage tissue. NPs sandwiched small RNAs with their nucleobase-derived nanotubes. In this study, we utilized NPs to deliver interleukin-1 receptor (IL-1R) siRNA into knee cartilage to prevent arthritis progression in a surgical induced PTOA mouse model. We injected fluorescent molecular beacons into knee joints of mice and determined the intracellular delivery using fluorescence molecular tomography (FMT). We also tested the therapeutic outcomes of IL-1R siRNA/NP in the destabilization of medial meniscus (DMM) mouse model. Results showed a significantly inhibition of cartilage erosion and arthritis progression using this treatment.

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**Eposterboard #25**

**AnEAR App Framework: Android Electronically Activated Recorder**

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Problem: There is a need for a readily-usable mobile application framework which can facilitate stable connections with wearable biosensors and storage, for healthcare research. We present the design and development of AnEAR (Android Electronically Activated Recorder), an application framework for the healthcare domain. AnEAR is designed for data collection, visualization, and analysis using wearable technology (WT), Internet of things (IoT), and deep learning (DL). This research tool expedites novel studies to improve outcomes by providing a robust smartphone application which features modular abstraction of communication with sensors, data storage, and analysis techniques. This allows researchers to integrate varied biosensor data without concern for the hardware specifics, helping to streamline connected
health research. Several experiments utilizing AnEAR are actively being conducted. In one study, AnEAR is used for collecting and visualizing continuous data from wearable biosensors (smartwatches and bands) of healthy individuals. This application is also being tested for collecting data from patients diagnosed with Post-traumatic stress disorder (PTSD), Autism, and Amyotrophic lateral sclerosis (ALS).

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Eposterboard #26

DEFINING THE TISSUE SPECIFIC AND SHARED DOWNSTREAM TARGETS OF FOXO TRANSCRIPTION FACTORS IN HUMANS

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Aging constitutes a major risk factor for several diseases such as cardiovascular and neurodegenerative diseases as well as cancer. Longevity and healthy aging are influenced by both genetic and environmental factors. Several studies have shown that FOXO transcription factors play a conserved role in aging and longevity. Conditions that increase FOXO activity can extend lifespan in worms, flies and mice. Intriguingly, recent genome-wide association studies have identified single nucleotide polymorphisms in the FOXO3 locus that correlate with longevity in humans. Despite many studies linking FOXO factors to longevity, how these factors directly regulate aging, particularly in humans, is not completely understood. In previous work, we found that FOXO factors regulate a core set target genes across different tissues in the mouse, as well as tissue-specific targets in different cell types. This study indicates that FOXO’s function differently in different cell types to promote cellular homeostasis in mammals, and highlight the need to define cell type specific targets in human cells. In our study, we will extend these findings to humans, and identify the shared and tissue specific downstream targets of three of the human FOXO’s: FOXO1, -3, and -4. To begin, we have identified antibodies suitable for immunocytochemistry in human cells, and are currently investigating their specificity in chromatin immunoprecipitation experiments. In parallel work, we are investigating how levels of FOXO proteins and activity change with age. Together, these studies will define the programs directly regulated by FOXO transcription factors in humans, and how they change with age.

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