2013 ABSTRACT BOOK

Summer Undergraduate Research Program

Graduate Programs
in the
Biomedical Sciences

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Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Division of Biomedical Sciences

SUMMER UNDERGRADUATE RESEARCH PROGRAM

2013

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Associate Dean for Graduate Programs
Director, Summer Undergraduate Research Program

2013 Summer Undergraduate Research Program

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Robin Berk	Cornell University	Dr. David Sharp
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Abstract

The Role of Myosins in the Formation of Phagocytic Cups During FcγR Mediated Phagocytosis in Macrophages

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Phagocytosis is a primary immune response by which macrophages engulf and destroy pathogenic, foreign particles and apoptotic cells in the human body. Several cellular pathways downstream of the major phagocytic receptor, the Fc-gamma receptor (FcγR), are involved in the engulfment process. During FcγR-mediated phagocytosis in macrophages, foreign cells coated with immunoglobin G (IgG) are recognized at the Fc domain, triggering the formation of phagocytic cups by actin polymerization. Phagosomes are formed once the cup-shaped invaginations of the plasma membrane close at their distal margins. A multitude of proteins implicated in the dynamics of the actin cytoskeleton are involved in this process. Myosin molecules, the only known actin-based motor proteins that responsible for contraction of actin filament networks, have been suggested to play a role in phagocytosis. Many different members of myosin family have been identified, however, which specific myosins are required and what precise step they play in the process still remains unknown. To examine the stated hypothesis, a macrophage cell line (RAW/LR5) was transfected with various shRNA plasmids, targeting specific myosin proteins and also coding for GFP. Phagocytosis assays using IgG-coated sheep erythrocytes followed by staining for actin and RBCs were performed for each construct to observe any changes in the phagocytosis pattern of positively transfected (GFP+) macrophage cells. Our preliminary screening has implicated several myosin in the process. Additionally, a preliminary experiment was performed to determine whether the identified myosin was required for extension of a pseudopod around the particle.

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Role of Liver in Chagas Disease

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Trypanosoma cruzi, an intracellular protozoan parasite that infects humans and other mammalian hosts, is the etiologic agent in Chagas disease. This parasite can invade a wide variety of mammalian cells including cardiomyocytes, adipocytes and hepatocytes. We have demonstrated that *T. cruzi* utilizes host LDL receptors to invade mammalian cells and that the rate of invasion depends on serum lipoprotein levels. Immunofluorescence analysis studies show accumulation of LDL/ cholesterol in organs such as heart, liver, and adipose tissue during acute and chronic infection. These observations suggest that infection alters systemic and whole body lipid homeostasis for parasite survival at different stages of infection. The liver plays a central role in whole body lipid homeostasis. Metabolic signals such as carbohydrates and dietary FAs regulate glycolytic and lipogenic signaling pathways. At the end of acute infection serum lipid levels are significantly decreased however, the liver could not replenish the serum lipid levels. Based on these observations we hypothesize that dysfunction of the liver contributes to the altered metabolic state during *T. cruzi* infection and may contribute to cardiomyopathy and metabolic disorders associated in Chagas disease. Also, diet plays an important role in maintaining liver functions during infection.

Methods: CD1 mice (8 weeks old, male) were fed on low fat diet (10%fat, RD), high fat diet (60% fat, HFD), RD with Lipitor (100mg/Kg body weight, RDL) or HFD with Lipitor (HFDL) for 30 days and then infected with trypomastigotes (Brazil $1x10^4$). Parasitemia, parasite load, Immunohistochemistry (IHC), Immunofluorescence analysis (IFA), Immunoblot analysis (IBA) etc. were performed using our established protocols. IHC and IBA data were quantified using NIH-Image J program and presented as bar graphs.

Results: Lipoprotein lipase (LPL), Uncoupling protein 3 (UCP3), Phosphatidylinositol (PIP2), ATP-binding cassette transporter (ABCA1) have shown an increase in expression while expression of Fatty acid synthase (FAS) has decreased when compared to uninfected mice. IBA shows that infected high fat diet fed mice have less macrophage infiltration when compared to infected regular diet fed mice.

Conclusions: *T. cruzi* has a high affinity to LDL and uses LDLr to enter the host cell. An explanation for the increase in LPL, UCP3, PIP2, ABCA1, is the high levels of cholesterol entering the liver during parasite infection. Excessive levels of fatty acids are shunted to the mitochondria for conversion into energy causing levels of UCP3 to increase as a response to mitochondrial stress. FAS enzyme levels are decreased in infected liver tissue, eventually fatty acid synthesis stops as hepatic tissue becomes necrotic. Immunoblot analysis shows an increase in inflammatory cells in hepatic blood vessels. Probing for the macrophage marker F4/80 shows that there is a substantial increase in macrophage presence in infected tissue. Interestingly, regular diet fed animals show a higher increase in macrophage presence when compared to high fat diet fed animals. This result correlates with the survival rate analysis. High fat diet mice survived 100% at the end of acute phase of infection while only 50% of regular diet fed animals survived. This suggests that a high fat diet may be preventing necrosis of hepatic tissue that is associated with inflammation. These results support the histological changes that occur in the liver after infection with *T. cruzil.*

Robin Berk
SURP Abstract
7/26/13

Title: Cep192: A Fundamental Centrosomal Activating Protein

Authors: Robin H. Berk, Brian P. O'Rourke, David J. Sharp

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Centrosomes are the primary microtubule nucleation and organizing centers in most eukaryotic cells. The pericentriolar matrix (PCM) surrounding the centrioles is directly related to specific functions of the centrosome. Here we study how depletion of a fundamental scaffolding PCM protein, Cep192, impacts the centrosomal localization of four other centrosomal proteins. Through siRNA transfection, immuno-staining, imaging, and intensity quantification, we assessed the level of the PCM proteins Gamma-tubulin, Cep192, Cep215, ALMS1, and Pericentrin in control and Cep192 depleted cells. Quantitative analysis shows Cep192 is nearly entirely depleted after siRNA treatment. The levels of Gamma-tubulin and Cep215 undergo a significant decrease after Cep192 depletion. In contrast, the levels of ALMS1 and Pericentrin significantly increase in Cep192 knockdown cells. Therefore, Cep192 differentially impacts the recruitment of specific PCM proteins, affecting the composition and function of the centrosome. These studies serve as a model in which specific activating proteins such as Cep192 can be brought to or removed from a centrosome with temporal regulation and drastically change the nature and capabilities of this organelle.

Gas6 Protects Against Axonal Damage in EAE

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A major goal of multiple sclerosis (MS)-related research is to identify factors that promote the repair and remyelination of MS lesions and protect against further damage. Our recent studies have been focused on how the protein, growth arrest-specific protein 6 (Gas6) is beneficial in alleviating inflammation and enhancing remeylination in animal models of MS such as cuprizone diet and MOG-induced EAE. As part of our long-term studies using these mouse models to characterize Gas6 signaling in the CNS, we administered Gas6, ACSF, and/or IFNβ to mice with MOG-induced EAE. Mice receiving Gas6 had significantly less severe courses of EAE compared to mice receiving ACSF only, and mice receiving IFNβ had a slightly delayed course of the disease. Immunohistochemical analysis of ventral spinal cords indicated a significantly reduced number of damaged axons in Gas6-treated mice. Additionally, we induced EAE in Gas6-/- mice, who had a significantly more severe course of EAE than wild type mice from days 16 to 21. However, there was no significant difference between amyloid precursor protein accumulations between Gas6-/- axons and wild type axons. Our results further support a role for Gas6 in the severity of deficits and axonal pathology in EAE.

Magnetic Resonance Imaging and Micro-Positron Emission Tomography Evaluation Effect of Diet on Chagas Heart Disease: Modulation of Dietary Fat as a Potential Therapy

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Approximately 7 to 8 million people suffer from Chagas disease, an infectious disease that can result in heart disease, caused by the *Tryopanosoma cruzi* parasite[1]. The disease begins with myocardial inflammation in the acute phase and progresses to severe cardiomyopathy in 30-40% of those in the chronic phase[2]. Accepted data now illustrates that adipose tissue acts as an early target and harbor for the *Trypanosoma cruzi* parasite[3]. This study aims to more comprehensively understand the relationship between the heart of those infected with Chagas disease and high fat diets so that this affinity can be manipulated to stabilize and contain the disease during its chronic stage. CD-1 mice were evaluated according to 4 variables: high fat diets, regular diets, variations in infection time and treatment with Lipitor (atorvastatin). The mice were imaged with magnetic resonance imaging (MRI) for anatomical and functional heart data as well as micro-positron emission tomography (micro-PET) for quantitative metabolic data. Use of the radioactive tracer ^{18}F-FDG allowed for quantitative analysis of the myocardium glucose metabolism. Measurements of heart dimensions (left and right ventricle inner dimensions, LVID and RVID) were achieved through analysis of MRI images with MATLAB and MIPAV computer programs. Results show a tendency for an increased ejection fraction in infected mice given a high fat diet. Additionally, infected mice fed high fat diets display slightly more normalized LVID measurements with wall sizes similar to the control, indicating that increased fat might act as a factor in developing a compensated cardiac hypertrophy and may delay progression to heart failure. Perhaps the most indicative results are the survival rates: regardless of drug treatment mice given high fat diets survived 92-100% of the time, as compared to a mere 46% survival rate with regular diets. These results signify potential clinical application of modulating fat content in the diet to prevent or retard progression of chagasic cardiomyopathy; however, larger mouse groups are required to establish more conclusive trends.

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Preclinical Evaluation of NETA-based Bifunctional Ligands for Radioimmunotherapy Applications using ^{225}Ac and ^{177}Lu : Radiolabeling, Serum Stability, and Biodistribution and Tumor Uptake Studies

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Abstract

Radioimmunotherapy (RIT) involves an antibody or peptide that is used to target a tumor, a radionuclide with therapeutic emissions, and a bifunctional ligand used to attach the radionuclide to the antibody.[1] Although targeted α-radioimmunotherapy (RIT), whose short penetration range is speculated to allow more selectivity in tumor targeting[1], has shown promising results in both preclinical and clinical trials and β-emitting radionuclides such as ^{177}Lu have been studied in detail for RIT, there is still progress to be made on evaluating chelators for RIT in general. As α-emitter ^{225}Ac's potency is shown through its apoptotic effects and its higher effectiveness per unit radioactivity than its daughter ^{213}Bi, the present study used it in radiolabeling of bifunctional chelators HS9-7c, HS11-8J, SX16-47, and SX15-66 as well as of 3p-*C*-NETA-Herceptin, 3p-*C*-NETA-Panitumumab, 3p-*C*-DEPA-Herceptin, 3p-*C*-DEPA-Panitumumab, and 3p-*C*-DECA-Herceptin were evaluated at pH 6.5 and room temperature[2,3]. *In vitro* stability of the ^{225}Ac-labeled conjugates was evaluated using human serum (pH 7, 37°C). An *in vivo* biodistribution study was performed to evaluate the *in vivo* stability and tumor targeting properties of the ^{225}Ac-3p-*C*-NETA-Herceptin and ^{225}Ac- *C*-DOTA-Herceptin conjugates in mice bearing tumors of the human LST174 carcinoma cell line. All ligands and conjugated antibodies (with the exception of 3p-*C*-DEPA-Panitumumab) were found to be rapid in complexing with ^{225}Ac though they did not exhibit stability in human serum. ^{177}Lu -3p-*C*-NETA-Herceptin demonstrated excellent *in vivo* stability as evidenced by low organ uptake and efficient tumor targeting that was favorably compared to those of ^{177}Lu-*C*-DOTA-trastuzumab, thus holding potential for RIT applications of ^{177}Lu.

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A High Throughput Approach to Anaerobic Structural Genomics with a Focus on Radical SAM Enzymes

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Radical SAMs are a diverse superfamily of enzymes categorized by their iron-sulfur [4Fe-4S] clusters typically anchored by three cysteine residues (motif: CxxxCxxC)[1]. The radical SAM superfamily, found in numerous organisms, including humans catalyzes the reductive cleavage of S-Adenosyl methionine (SAM), generating methionine and a 5′-deoxyadenosyl 5′-radical. These proteins are involved in a number of chemical reactions, including methyl transfer, biotin and lipoic acid synthesis as well as the electron transport chain[2]. Currently there is limited functional information for radical SAM enzymes, but recent findings indicate a number of potential medical implications, including bacterial[3] and viral defenses[4]. Studying the structure of radical SAMs could allow for a better understanding of their diverse functions, though it is complicated by the fact that they are oxygen labile. To accommodate this, the production, purification and crystallizations are all performed via high-throughput methods under strict anaerobic conditions via a nitrogen-filled glove box.

Radical SAM targets are first cloned with an affinity tag into an expression plasmid, then transformed and overexpressed in *E. coli* (BL21 [DE3]). They are purified using affinity chromatography at the small-scale level and their expression is evaluated via an SDS-PAGE gel. Targets that express well are grown in larger quantities and a size-exclusion column is added to the purification. Once purified, the proteins are set up in crystal trays, with the hopes that diffraction-worthy crystals will grow. Crystals are exposed to x-rays and their diffraction patterns analyzed to yield an electron density map and crystal structure.

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Modification of Antibody 2556 Recognizing HIV Protein gp41 with CHXA" Ligand for Radiolabeling and Radioimmunotherapy

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The survival of patients with HIV has been successfully prolonged due to antiretroviral therapy. However, the blood brain barrier (BBB) decreases the permeability of many drugs into the central nervous system. As a result, the brain can become infected, leading to neurocognitive disorders such as HIV associated dementia. Previous research showed that radioimmunotherapy (RIT) targeting the HIV gp41 glycoprotein successfully kills infected HIV cells and virions, eliminating HIV in tissue culture cells and in mice. Here we examined the ability of 2556, a gp41-specific radiolabeled monoclonal antibody, to take up the ligand CHXA" (*trans*-cyclohexyldiethylenetriamine) at different molar ratios, and tested to see if 2556-CHXA" was able to maintain its immunoreactivity to gp41, using a direct ELISA assay. We were successful in chelating 2556 to the ligand CHXA". A starting molar excess of 50X CHXA" yielded 6X final molar excess of CHXA" attached to 2556, which retained about 30% of its original immunoreactivity. A starting molar excess of 2X yielded a final molar excess of 1X and 100% of the original immunoreactivity. We concluded that the lower the ratio of CHXA" to 2556 the more immunoreactivity the antibody retains. Ultimately, we will radiolabel the 2556-CHXA" complex with Bismuth to see how well the protein retains immunoreactivity, and we will examine 2556 and see if the protein's isoelectric point has been altered thus enabling it to easily enter through the BBB.

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The generation of specialized transducing phage for the creation of Δ*mbt1 Mycobacterium tuberculosis* mutants defective in iron uptake

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Abstract: *Mycobacterium tuberculosis* is an extraordinarily well-adapted human pathogen, infecting approximately one third of the world's population. The bacterium relies on small, organic molecules called mycobactins to acquire iron from the restrictive environment of its preferred host, human macrophages. Mycobactins have significantly higher iron binding affinity than host iron binding proteins and work by cyclically shuttling the essential metal into the bacteria. Preliminary data have shown that in iron uptake assays in which radiolabeled mycobactin was added to iron uptake mutants, significantly lower internal mycobactin concentrations were observed as compared to the wild type; however, analysis remains ambiguous due to endogenous production of mycobactin. The goal of this project aims to eliminate mycobactin synthesis in these mutants to create more definitive uptake assays. To this end, here we describe our process of generating a specialized transducing phage to knock out the *mbt1* genetic locus, which is responsible for endogenous production of mycobactins. This technique relies on the chimeric nature of the phasmid, which replicates as a plasmid in *E. coli* and as a bacteriophage in *Mycobacterium* species. The use of specialized transducing phage ensures the highly efficient genetic manipulation and delivery of an allelic exchange substrate (AES) into *M. tuberculosis*. This AES consists of a selection cassette flanked by sequences homologous to the periphery of the original *mbt1* locus, and will replace the locus by homologous recombination once introduced to the mycobacteria. The successful PCR construction of the allelic exchange substrate, its ligation into the specialized transducing phasmid, and the generation of a high titer lysate have been accomplished thus far.

Visualization of Primary Cilium Using Correlated Fluorescence and Interference Microscopy

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The primary cilium has long been incorrectly considered to be a vestigial organelle. Although the primary cilium was observed in the late 1800's and identified by electron microscopy in the 1950's, experimental investigations of the primary cilium and ciliopathies have experienced a renaissance. Unlike the cilia found in the human trachea, there is only one primary cilium per cell and because it lacks a central pair of microtubules, it is immotile. Nevertheless, the primary cilium is essential to a plethora of sensory functions and cell signaling pathways. Disruption of the Intraflagellar Transport (IFT) genes, which are essential to properly building the primary cilium, causes polycystic kidney disease. We seek to perturb the direction of the primary cilium in a cell, and therefore require a method for its real-time visualization.

We have implemented immunostaining, GFP visualization, and label-free imaging of the primary cilium in fixed and live cells of three cell types: (1) Mouse kidney epithelial cells; (2) Mouse Embryo Fibroblasts (MEF) and (3) FlpIn-3T3 fibroblasts. We have quantified the frequency and shape of the primary cilium under various conditions: (1) wild-types; (2) IFT-88 knockouts; (3) serum-loading and (4) duration of starvation.

Inversin-GFP enables real-time imaging of the primary cilium and its microtubule-dense structure provides a strong signal for label-free imaging using a form of interference microscopy that we call Laser Feedback Microscopy. Our work lays the foundation for real-time perturbation of the primary cilium that will enable us to study the responses of the PDGF-AA signaling pathway during chemotaxis.

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Exploration of Differences in LacNAcylation Between Naïve and Active T-Cells

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The *in vitro* imaging of higher-order glycans has recently been enabled using a chemoenzymatic strategy. An azide- or alkyne-bearing monosaccharide is transferred enzymatically to the target glycan on the cell surface. Click chemistry can be utilized to fluorescently label the tagged cells in the subsequent step. In our previous studies, it was shown that naïve t-cells have less accessible *N*-acetyllactosamine (LacNAc) than active t-cells. This project was developed to determine whether the observed difference could be attributed to LacNAc labeling being blocked by other monosaccharides or to the absence of LacNAc altogether. CD4+ t-cells were isolated from mouse spleens, costained for LacNAc and other glycan epitopes by lectins and a marker of activation, CD44, and analyzed by flow cytometry. Lectin staining showed that both terminal Neu5Ac and terminal Neu5Gc were present in CD4+/CD44- and colocalized with LacNAc- cells, suggesting that sialic acids may be blocking LacNAc expression. Terminal galactose was mostly absent. T-cells were then treated with Neuraminidase to cleave off terminal sialic acid residues and analyzed with lectin staining and flow cytometry, resulting in increased LacNAc labeling; thus, Neu5Ac is at least partially blocking LacNAc expression. Future experiments include a proteomics analysis of isolated t-cells as well as possible treatment with a sialidase to cleave off terminal Neu5Gc. Funding was provided by the Graduate Division of Albert Einstein College of Medicine.

Methylation-Regulated Biomarkers for Radiosensitivity in Head and Neck Squamous Cell Carcinoma

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Background: Over 52,000 people are diagnosed in the United States with head and neck squamous cell carcinoma (HNSCC) each year. Most patients will receive radiation therapy (RT) as either primary or adjuvant treatment. There are currently no molecular biomarkers used to predict who will respond. Preliminary work by our group has identified a series of genes that are differentially methylated between patients with HNSCC that either remained disease-free or recurred after primary RT.

Objective: The goal of this research is to identify differentially methylated genes that can be used to predict response to RT in HNSCC cells.

Methods: Illumina Infinium 27k methylation arrays (Illumina®, San Diego, CA) were performed on 13 tumor-normal pairs from patients with HNSCC that received primary RT. Patients were divided into those that either remained disease-free for 24 months (9), or those that experienced locoregional failure (4). Differentially methylated genes were identified. We then examined the radiosensitivity of 8 HPV-negative HNSCC cell lines via clonogenic assay. We also performed western blot analysis to detect the expression of CDH9, one of the differentially methylated genes identified in our discovery cohort.

Results: Our characterization of these lines demonstrated a range of sensitivities radiation. CDH9 expression may be associated with radioresistance.

Discussion: We have determined that HNSCC lines demonstrate a range of sensitivities to external beam radiation. Exploration of specific genes related to these observed differences is ongoing. Future work will further explore CDH9 and other potential targets.

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Kinetic Characterization of L-Aspartate Oxidase from *Mycobacterium tuberculosis*

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Abstract

The recent appearance of drug-resistant strains of *Mycobacterium tuberculosis* has necessitated efforts to develop new, more effective treatments for tuberculosis. Previous research has suggested that the enzymes involved in bacterial NAD^+ biosynthesis could potentially be good targets for inhibition. The present study focused on determination of the kinetics and mechanism of the first enzyme in the *de novo* NAD^+ biosynthetic pathway in *M. tuberculosis*, L-aspartate oxidase (NadB), the function of which is to oxidize L-aspartic acid to iminoaspartate. The enzyme was expressed in *Mycobacterium smegmatis*, purified, and used to perform enzymatic activity assays. Recombinant NadB displayed a V_{max} of 2.07 ± 0.04 min^{-1} and a K_m of 1.07 ± 0.09 for aspartic acid *in vitro*. The pH-rate profile was bell-shaped with pK_a = 6.64 ± 0.04 and pK_b = 9.71 ± 0.04, indicating an acid-base mediated catalysis. These results are in agreement with the finding that the highly conserved E121 and R281 are directly involved in catalysis and binding. The observed SKIEs of $E_{V/K}$ = 2.23 ± 0.32 and E_V = 1.32 ± 0.07 suggest that the chemistry of the first half-reaction is potentially rate limiting. While the results from the SKIE experiment may also support a mechanism involving hydrogen abstraction from the N-terminus of L-aspartate, primary and multiple kinetic isotope effect studies are required to confirm this hypothesis.

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The Cues That Shape Protective Immunity

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Vaccines confer improved protection against microbial diseases through the induction of long-lived memory T and B cells in vaccinated individuals. Strong $CD8^+$ T cell memory is important for long-term protection against intracellular pathogens. Memory T cells are not homogenous; they include different populations with distinct phenotypes and functions. However, how this heterogeneity relates to their functions is not fully understood. We used mice immunized with *Listeria monocytogenes* (*Lm*) as a model, which develop memory $CD8^+$ T cells that confer life-long protection to vaccinated hosts. Previous work in the lab has characterized mutant strains of *Lm* that fail to confer protection to immunized hosts, and correlated the presence of memory $CD8^+$ T cells that express the cell-surface lectin KLRG1 with protection against a challenge infection with WT *Lm*. Of note, the $KLRG1^+$ subset of memory $CD8^+$ T cells did not express CXCR3, a chemotactic receptor involved in T cell recruitment to infected tissues. My SURP project was to study the phenotype, function and formation of $KLRG1^+CXCR3^-$ and $CXCR3^+KLRG1^-$ memory $CD8^+$ T cell-subsets after vaccination with *Lm*. We showed that $KLRG1^+CXCR3^-$ memory $CD8^+$ T cells exhibit an effector phenotype, localized to non-lymphoid tissues and are found in greater numbers in protected mice. In contrast, $CXCR3^+KLRG1^-$ cells express more memory markers and are highly represented in lymphoid organs. We also found that interleukin 12 along with IFNγ, promote the onset of $KLRG1^+$ memory cells. Finally, we highlighted that inflammatory monocytes may be critical in determining the ratio between these two subsets of memory cells.

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Exploring the role of protein synthesis in long-term potentiation expressed by NMDA receptors in the dentate gyrus

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Excitatory synaptic transmission is typically mediated by AMPA and NMDA ionotropic glutamate receptors. While AMPA receptors (AMPARs) mediate most fast excitatory transmission, NMDA receptors (NMDARs) play a crucial role as triggers of long-term synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) of AMPAR-mediated transmission. LTP/LTD are the best known cellular correlates of learning and memory. Growing evidence indicates that NMDARs also express LTP and LTD, but the mechanisms underlying these forms of plasticity remain poorly understood. For example, while it is known that protein synthesis is required for AMPAR-LTP, such a requirement has yet to be determined for NMDA-LTP. Here, using acute rat hippocampal slices, we tested the possibility that NMDAR-LTP requires protein synthesis for its expression. To this end, we recorded isolated NMDAR-mediated transmission in the medial perforant path of the dentate gyrus in rats before and after bath application of cycloheximide, a specific translation inhibitor. We found that NMDAR-LTP was insensitive to the block of translation, suggesting that protein synthesis is not required for its expression. To confirm the efficacy of cycloheximide, we recorded metabotropic glutamate receptor (mGluR)-dependent LTD in the CA1 region of the hippocampus, a phenomenon known to require protein synthesis. Consistent with previous findings, cycloheximide completely abolished agonist-induced mGluR-LTD. Thus, unlike AMPAR-LTP, the expression of NMDAR-LTP in the dentate gyrus is protein synthesis-independent. NMDAR-LTP seems to be a post-translational phenomenon. Further investigation into the functional consequences of this form of plasticity may have important implications on our understanding of learning and memory.

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FEZ1-dependent inhibition of autophagy is regulated by metabotropic glutamate receptors

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The breakdown of long-lived proteins by autophagy is a pathway imperative to cell survival. Consequently, both elevated and insufficient levels of autophagy have been shown to lead to neurodegeneration. Recent studies have sought to elucidate the underlying mechanisms involved in autophagy in order to better understand the pathway as a whole. A study by McNight *et al.* (2012) identified SCOC as a protein required for autophagosome formation, and reported that SCOC forms a complex with Fasciculation and Elongation protein Zeta 1 (FEZ1) under starvation conditions.

Here, we assess the relationship between FEZ1 and the GPCR metabotropic glutamate receptor 1 (mGlu1) in the modulation of autophagy. As FEZ1 interacts with mGlu1 at its carboxyl-terminal domain (unpublished results), we aimed to visualize the localization of these proteins in relation to LC3, a cytosolic (LC3-I) protein that is conjugated to phosphatidylethanolamine (LC3-II form) and recruited to the autophagosomal membrane during autophagy. Quantification of LC3-I and LC3-II levels allowed us to monitor autophagic flux in cells in which FEZ1 and mGlu1 were overexpressed. Furthermore, treatment with the mTOR inhibitor rapamycin was used to enhance autophagy, as high mTOR activity prevents autophagy induction under conditions of nutrient sufficiency. Cells were also treated with the mGlu1 selective agonist DHPG in order to further explore the impact of the metabotropic pathway on autophagy. Preliminary findings indicate that FEZ1 overexpression in COS7 cells may cause suppression of autophagic activity. Our observations further suggest that the capacity of FEZ1 to suppress autophagy is impaired in cells co-expressing mGlu1, thus pointing to a functional link between metabotropic activity and the autophagy pathway.

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Small Molecule Targeting of Antiapoptotic MCL-1

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Apoptosis, or programmed cell death, is the natural process by which specific cells within an organism are destroyed to aid in development or to prevent disease. Apoptosis is suppressed by the antiapoptotic members of the B-cell lymphoma 2 (BCL-2) family of proteins, such as myeloid cell leukemia 1 (MCL-1), which contributes to cancer cell survival leading to tumorigenesis, tumor maintenance and chemoresistance. Recently, attention has turned to MCL-1 as a potential avenue towards combating cancer by inhibiting this particular antiapoptotic protein, since MCL-1 is overexpressed in a number of human cancers. Understanding how small molecule inhibitors bind to and suppress MCL-1 is an essential means to uncovering the key factors in MCL-1 inhibition and to developing more effective drugs to target MCL-1 *in vivo*. Here, using derivatives of the first-generation MCL-1 inhibitor, maritoclax, and chemical and structural biology approaches, we have begun down the path towards unveiling the critical structural characteristics of small molecule MCL-1 inhibitors. Our long-term goal is to develop a potent and selective MCL-1 inhibitor to probe the function of MCL-1 in tumor survival and to potentially use in development of novel anticancer therapeutics.

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Development of a Listeria-poliovirus Cancer Vaccine

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The use of foreign antigens in cancer immunotherapy has been shown to induce

greater $CD4^+$ and $CD8^+$ T cell responses than self or mutated antigens, providing a novel solution to low immune responses against cancer vaccines in the past. The goal of this project was to construct a vaccine against metastasic breast cancer by cloning a poliovirus (PV) antigen into a *Listeria monocytogenes* (LM) vector. The Listeria vector (LM-LLO) contains Listeriolysin O (LLO), a secreted pore-forming toxin, which will ultimately be fused with PV to make a recombinant protein. The LM-LLO vector and PV insert were isolated, purified, and ligated together. The subsequent plasmid was then used to transform *E.coli*. The presence of PV insert in the LM-LLO plasmid will be confirmed by restriction enzyme digestion and DNA sequencing. Work is in progress to obtain a colony with the LM-LLO-PV plasmid, after which it will be introduced into Listeria through electroporation. When the presence of the poliovirus antigen is confirmed through western blot analysis, LM-LLO-PV will be ready to be tested in a mouse model with breast cancer.

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Optimizing Cryopreservation Procedure of Hematopoietic Progenitor Cells

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Hematopoietic progenitor cell (HPC) transplantation following high-dose chemotherapy is used primarily for therapy of hematologic malignancies. Improvements in the HPC cryopreservation procedure are necessary to maximize therapeutic outcome. Standard operating procedures (SOP) utilize 10% dimethyl sulfoxide (DMSO), 10% anticoagulant citrate dextrose (ACD-A), and 30% plasma for cryopreservation. There is a variety of accepted published protocols showing that a mixture of 6% hetastarch (HES) final concentration to DMSO improves transplant outcome. However HES is not commercially available in the required concentration. HPC products diluted post-thaw with Dextran-40 also increases total cell viability. We hypothesize that the addition of low concentration HES to the current cryopreservation protocol, combined with a post-thaw dilution with Dextran-40, will further increase total cell viability of HPC products. HPC products collected by apheresis were cryopreserved in both standard and protocols including up to 3% HES and different Albumin concentrations. Products stored below -80^{o}C to -196^{o}C were split post-thaw in Dextran-40 diluted and undiluted conditions. Cell viability was determined by trypan blue staining. Our experiments indicate that standard 10% DMSO cryoprotectant with Dextran-40 1:1 post-thaw dilution yielded the highest post-thaw cell viability, and was superior to protocols with addition of <3% HES under both undiluted and diluted conditions. Future investigations will focus on further optimization of cryopreservation procedures.

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The Role of Cx43 Gap Junction Channels in Propagation of Cell Death

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Connexins (Cxs) are membrane proteins that play a role in intercellular communication of vertebrates. Currently 21 human Cx isoforms and 19 mouse Cx isoforms have been identified (Sohl and Willecke, 2003). Cxs have four alpha helical transmembrane domains (TM1 to TM4), intracellular N- and C-termini, two extracellular loops, and a cytoplasmic loop (Sosinsky and Nicholson, 2005). Six Connexin-subunits oligomerize to form a hemichannel. Hemichannels then modulate membrane permeability under specific physiological and pathological situations or form a jap junction (GJ) by docking with an apposing hemichannel on an adjacent cell. Clustering of GJ leads to the formation of a junctional plaque (JP), facilitating electrical intercellular communication, exchange of metabolites and small ions below 1000 Da, such as NAD^+ and ATP. Most vertebrate cells in the fully differentiated state express Cxs, with the exception of red blood cells, spermatozoa, and skeletal muscle (Saez et al., 2003).

GJs have also been linked to the cell-to-cell spread of agents causing apoptotic or necrotic transformation of neighboring cells (Decrock et al., 2009). Alternatively, intercellular exchange through GJs may prevent the accumulation of necrotic or apoptotic factors, such as oxidative radicals, reducing ionic misbalance, etc. (Lin et al., 1998). Therefore, in the current study, necrosis was induced in HeLa cells expressing Cx43 fused with enhanced green fluorescent protein (EGFP) at the C-terminal. Cell death was generated in individual cells by exposure to focused Ultra Violet (UV) light at 5.0 mV. The rate of necrosis/apoptosis progression was determined by measuring fluorescence intensity emitted from Propidium iodide (PI) and 4',6-diamidino-2-phenylindole (DAPI) dyes absorbed into the cell nucleus.

Rate of cell death propagation was observed in the UV exposed cell and neighboring cells in the presence and absence of GJPs. We expected that necrosis would occur at a higher rate in UV exposed cells not expressing GJs and the spread of cell death to adjacent cells would not be significant. Conversely, the rate of necrosis development in UV exposed cells would be lower in the presence of GJs; but the spread of death to neighboring cells connected through GJs would increase. It's possible that the accumulation of necrotic/apoptotic factors in the UV exposed cell that could ultimately result in the cell's death, may be alleviated by transfer of such agents through GJs into healthier adjacent cells. Thus, GJs may play a role in preventing the build up of necrotic/apoptotic metabolites resembling processes occurring in tissues under pathological conditions such as ischemia or stroke.

Targeting and killing HIV-infected cells with antibody-toxin conjugates

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July 26, 2013

Candice Church Harris Goldstein

Albert Einstein College of Medicine

Abstract

Gelonin is a naturally occurring protein derived from seeds of the Himalayan plant *Gelonium multiflorum*. A homologue of ricin (famed for its appearance on certain letters to the Hill as well as in Breaking Bad), gelonin acts by the same mechanism, irreversibly hydrolyzing a N-glycosidic bond within the 28S rRNA, preventing protein synthesis and causing cell death.

A recombinant gelonin, showing improved pharmacodynamics in vivo, has been shown to be effective in inhibiting HIV infection as well as replication in already infected cells. Compared to ricin, gelonin's toxicity is much easier to control because it cannot enter healthy cells by itself. To achieve specificity and target HIV-infected cells with rGel, we conjugated it to a broadly neutralizing monoclonal antibody, VRC01. Binding to gp120, a glycoprotein exposed on the surface of the HIV envelope, VRC01 is capable of neutralizing 90% of HIV strains. Similar recombinant fusion constructs have demonstrated potent cytotoxic activity *in vivo* against melanoma. We explore the potential for these conjugates to target and kill HIV-infected cells in this project.

Title:
Phagocytosis of *Cryptococcus neoformans* by Murine Macrophages

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Abstract

Cryptococcus neoformans (*Cn*) is a facultative intracellular pathogen that causes life threatening pneumonia and meningoenchephalitis in immunocompromised patients. It has been shown that *Cn* is capable of efficient replication within host lung macrophages and that it can survive as a latent form within lung macrophages for years. Thus, macrophage phagocytosis of *Cn* is an effector mechanism of the host immune response but might also facilitate pathogen latency. In this study, three different types of murine macrophages where examined: J774.16 tumoral macrophage cell line, bone marrow derived macrophages (BMDM), and peritoneal macrophages. Macrophages were infected for 24 and 48 hour periods and examined for: macrophage number through propidium iodide staining, total ATP content, and yeast killing assays through Colony Forming Units (CFU). We found that *Cn* does not kill J774.16, BMDM, nor peritoneal cells, but there is a decrease in ATP levels in J774.16 and peritoneal macrophages. BMDM cells ATP levels seemed unaffected after 24 hour infection period, but at 48h one can observe a decrease in ATP. J774.16 and Peritoneal macrophages had a decrease in ATP as early as 24 hours. At 24h infection, all macrophages were able to restrict *Cn* growth, but this ability was lost when infection was prolonged to 48h. In preparation for in vivo studies of cryptococcal infection, we developed a fluorescence activated cell sorting (FACS) protocol to characterize immune cells of the lung and spleen. The results from this project will foster understanding of cryptoccocal disease mechanisms with the goal of improving clinical outcome for this pathogen.

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Preliminary Explorations into Bacterial Swarming

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The relationship between bacterial motility and disease is poorly understood. Preliminary evidence suggests extrinsic factors from the host, such as mucins, can alter motility and biofilm formation. In an effort to understand changes in bacterial motility during states of human pathology, when host secretions change from their homeostatic balance, we exposed *Bacillus subtilus* to enterotoxins obtained from colitic mice. Our preliminary results indicate that one or more enterotoxins inhibit bacterial swarming. The consequences of hindered swarming may be either beneficial to the host, serving a protective effect, or harmful, allowing for greater pathology and enteroinvasiveness of the bacteria. Future studies will focus on optimizing assays and isolating various components of host secretions. Thank you to SURP and Dr. Kearns' and Dr. Mani's labs for their generous support and guidance.

Calcium signaling between sensory neurons and glia in orofacial pain

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The trigeminal ganglion (TG) plays a prominent role in facial nociception. Satellite glial cells (SGCs) in TG surround neuronal cell bodies and communicate with one another and with neurons through intercellular calcium waves (ICWs). ICWs can be transmitted via gap junctions or via a pathway involving ATP release through pannexin1 (Panx1) channels and the activation of purinergic receptors (P2R). This type of communication under pain conditions has not been explored *in vivo*. We therefore analyzed Ca^{2+}-communication between sensory neurons and SGCs in a mouse model of chronic orofacial pain using mice that genetically express the Ca^{2+}-indicator GCaMP3 in either neurons or glia. Intact TG were stimulated using ATP to activate P2Rs or K^+ to activate Panx1. We observed higher baseline Ca^{2+} activity and reduced thresholds to ATP and K^+ in neurons and SGC of TG from the pain model. In addition, 1) the amplitude of neuronal responses increased, with higher sensitivity to K^+; 2) SGC responses showed higher frequency, but no amplitude change. Preliminary data show reduced Ca^{2+}-signaling after treatment with the broad-spectrum P2R antagonist suramin, the gap junction/Panx1 blocker carbenoxolone, or with Panx1 blockers probenecid and mefloquine. These results indicate that Ca^{2+}-signaling between neurons and SGCs in sensory ganglia is intensified under pain conditions and is mediated by a pathway involving the activation of P2Rs and Pannexin1, and to some extent by gap junctions. Increased glial-neuronal Ca^{2+} signaling in allodynia likely plays a role in the neuronal hyperexcitability within sensory ganglia associated with our orofacial pain model.

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Characterizing the effect of PfENT1 single nucleotide polymorphisms on the Efficacy of PfENT1 Inhibitors

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Malaria is an infectious disease caused by parasites of the genus *Plasmodium*. Malaria is an important global health problem with high morbidity and mortality rates. Due to the emergence of resistance to current antimalarial drugs, it is important to establish new drug targets. During the parasite's 48 hr life cycle, it duplicates its DNA 8-32 times. Like many other protozoan parasites, *Plasmodium* lacks *de novo* purine synthesis. It relies on purines scavenged from its host, making the import pathway a potential drug target. Purine salvage pathway enzymes process the imported purines.to those needed for DNA synthesis and metabolic processes The parasites have four equilibrative nucleoside transporters (ENTs) that allow purines to go down their concentration gradient from the host red blood cell and into the parasite cytoplasm. The primary ENT is *P. falciparum* equilibrative nucleoside transporter 1 (PfENT1). We examined the effect of four naturally occurring PfENT1 single nucleotide polymorphisms (SNP) on adenosine transport and on the efficacy of recently identified PfENT1 inhibitors. We expressed codon-normalized PfENT1 and the four SNPs in purine auxotrophic *ade2* knockout yeast. We characterized the dependence of growth on the media adenosine concentration. We show that compared to WT PfENT1, these four SNPs do not cause major changes in yeast growth rates. We also investigated the effect of these mutations on the IC_{50} of PfENT1 inhibitors. None of the mutations significantly increased the inhibitors' IC_{50} compared to WT PfENT1. This suggests that these naturally occurring mutations would not cause resistance to these PfENT1 inhibitors.

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The Role of the Neck Linker in Kinesin-13 Microtubule Depolymerization

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The structural basis of the mechanism of kinesin-13s microtubule (MT) depolymerizing protein is not well understood. Like other kinesins, kinesin-13s have a motor-head domain (HD) with an ATP and tubulin binding site. Previous studies have shown that the motor domain is sufficient to induce MT depolymerization but a stretch of positively charged residues N-terminal of the HD, the "neck", is required for normal depolymerization activity in-*vivo*. To elucidate the role of the neck we analyzed protein constructs of the *Drosophila melanogaster* kinesin-13s, KLP10A containing the HD and neck (KLP10AMN), the motor domain alone (KLP10AHD), or the neck alone. *In vitro* polymerization assays showed that both KLP10AHD and KLP10MN depolymerize MTs. However, KLP10MN showed a higher rate of depolymerization. The neck construct alone showed no MT depolymerization activity. These results indicate that the neck potentiates the depolymerization activity of the motor domain. We then used electron microscopy (EM) to investigate the structural basis of this neck potentiating effect. Tubulin incubated with KLP10AHD or KLP10ANM formed ring complexes consisting of curved tubulin protofilaments with bound kinesin HD. The curvature of the rings was similar in both cases, ruling out an effect of the neck on tubulin curvature. However, in the presence of AMPPNP (non-hydrolysable ATP analogue) KLP10AMN would bind to every other tubulin heterodimer instead of every heterodimer like KLP10AHD. This pattern was dependant on the nucleotide state. In the presence of ADP-AlF_4^- (ADP-Pi transition state mimic), both KLP10HD and KLP10MD constructs bind to every tubulin heterodimer. This result indicates that the nucleotide species in the ATPase site of the HD controls the position or conformation of the neck.

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Point of Care Rapid-HIV Testing in New York City Public Schools: An Analysis of Cost, Benefit and Utility

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Montefiore Medical Center provides medical care through the school based health program for 20 high schools and middle schools in the Bronx. Amongst these are 13 schools participating in the Point of Care Rapid-HIV program. This analysis determines the cost, benefit and utility of the POC OraQuick Advance HIV-1/2 Rapid Antibody Testing (HIV-R) as compared to standard in lab testing (HIV-IL) in the New York City public school system. Pre-analytic, analytic and post-analytic variables were considered. The cost effectiveness of HIV-R as compared to HIV-IL was found to be dependent upon the personnel performing and reviewing the test, volume of tests/month, and amount of time spent reviewing results. Based on cost alone, when testing volume exceeds 20 tests/month, HIV-R is generally cost effective as compared to HIV-IL. Students receive their HIV-R results within 27.3 min. However, students can wait several days to receive their HIV-IL results due to scheduling issues. Providers reported a dramatic increase in consent rates when switching to HIV-R, with many students consenting when learning of the test's non-invasive nature. Additionally, students' desire for HIV testing is often couched under the guise of a check-up. According to providers, this may be due to a stigma attached to directly requesting an HIV test. We found that one way to increase testing in low volume high schools is to have an HIV awareness day where HIV-R is offered a few times annually. However, throughout the year HIV-IL only would be offered. This preliminary analysis creates a model that is easily translatable to new POC testing as it becomes available for other infectious diseases. The school based health program is a natural pilot environment for newly developed POC testing. I would like to thank SURP at AECOM for funding this project and the entire POC Division of the Department of Pathology for their help and support.

Effect of Heat Inactivation on Serum Antibody Reactivity Against Mycobacterial Antigens

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Background: When studying antibodies (Abs) in human samples it is critical to deactivate blood-borne pathogens such as HIV or other viruses to avoid the risk of exposure. Common methods to deactivate viruses are heat inactivation or incubation with Triton X. While treatment with Triton X can be cumbersome and time-consuming, heat inactivation is more practical and time-efficient. In general, heat inactivation is believed to have little impact on the structure or function of Abs although some studies have generated controversial results. The objective of this study was to investigate the impact of heat compared to Triton X or no treatment on the serum Ab reactivity against different mycobacterial antigens, two proteins and a polysaccharide.

Methods: Serum samples (n=38) from HIV- patients with and without active tuberculosis (TB) were tested by ELISA for IgG, IgG subclasses, IgA and IgM against the two immunodominant mycobacterial proteins MPT51 and malate synthase (MS), and the capsular polysaccharide arabinomannan (AM). The 96 well plates were coated with antigens and then blocked with either 3% BSA or 1% milk. Sera were untreated or inactivated for infectious agents with either 1% Triton X-100 or heating at 56°C for 30 minutes and diluted 1:50 in 0.3% BSA or 0.1% milk. We further evaluated the effect of heat on several murine monoclonal Abs (mAbs).

Results: IgG reactivity against the two proteins MS and MPT51 was significantly increased in the heat treated versus untreated samples for both TB+ and TB- control subjects ($p<0.0001$). By contrast, no clinically meaningful differences in IgG reactivity against the proteins were observed between Triton X and untreated samples. The increased IgG reactivity of the heat treated samples, which with several samples was further increased when blocking milk with 1% milk instead of 3% BSA ($p=0.02$) was reversed when adding 0.1 % milk instead of 0.3 % BSA to the serum dilution buffer. No Ab reactivity was observed when plates lacked proteins and were only blocked with 3% BSA. Interestingly, no significant influence of heat was detected for IgG reactivity against AM. Significantly increased reactivity of the heat treated compared to untreated samples against MPT51 was also observed for IgA and IgM but to a lesser extent ($p<0.0001$). Furthermore, heat treatment also increased the reactivity of the mAbs 3EF (IgG1 and IgG3), 16A1 (IgG1) and 16A6 (IgG1) against MPT51 and glucuronoxylomannan (GXM) while it had no effect on some other mAbs such as 8F3 (IgG1) and 10F4 (IgG1) against edema factor (EF) and (PA).

Conclusions: Heat treatment of sera leads to an increased Ab reactivity that is non-specific, depends on the target antigen, and affects certain Ab isotypes and IgG subclasses more than others. Thus, heat inactivation should be avoided in serological studies.

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Discovery of small molecule biomarkers for the rapid diagnosis of bacteremia

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Bacteremia is a leading cause of mortality in hospitalized patients. The presently available diagnostic test requires 24-48 hrs for final identification. To develop a rapid, point of care (POC) diagnostic we have carried out global metabolite profiling on patient plasma in bacteremic versus non bacteremic hospitalized patients from Jacobi Medical Center. Metabolites were extracted and analyzed with a UPLC system coupled with a quadrupole–time of flight hybrid mass spectrometer and analyzed using specialized software. We compared plasma collected at the time of blood cultures sampling in 19 bacteremic and 21 non bacteremic samples. Patient clinical and laboratory characteristics were similar between each group. Based on partial least squared discriminatory analyses (PLS-DA), we found good segregation between each groups. A total of 23 markers were different between bacteremic and sterile groups (Mann-Whitney U test, $p<0.05$, FC > 2.0). We also identified molecules that were significantly different between Gram positive and Gram negative samples and between MSSA and MRSA. These data provide novel discoveries of the metabolomic responses of the host and pathogen in vivo during infection and potential biomarkers that could be developed as a POC test to improve clinical care.

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The Role of Cancer-Derived p53 in Recruiting the TFIID Complex To Initiate Transcription of p53 Target Genes

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Various mechanisms are involved in the regulation of DNA transcription. Sequence-specific DNA-binding activators have been found to stimulate transcription, at least in part, by initiating the binding of the TFIID complex to the DNA promoter region. TFIID is a transcription factor that binds to the TATA box and recruits other proteins important for transcription initiation, such as RNA polymerase II, to the promoter. One activator, which recruits the binding of TFIID to the DNA, is the tumor suppressor p53 protein. P53 is widely studied as it is an important factor in regulation of the cell cycle. DNA damage and other types of cellular stresses cause p53 to activate transcription of target genes, which act to maintain genome integrity through cellular responses such as growth arrest, DNA repair, and apoptosis. Certain mutations in the p53 sequence are therefore a leading cause of cancer, as cell regulation deteriorates. The p53 mutants most commonly found in tumors, known as "hotspot mutants," are p53-R248Q, p53-R273H, and p53-R175H. Interestingly, these mutations are located in the DNA-binding sequence of the p53 protein. Previous research has shown that wild type p53 is involved in the binding of the TFIID complex to the DNA promoter. However, it is unknown whether cancer-derived p53, with mutations in the DNA-binding sequence, interacts differently with the TFIID complex and the DNA of target genes. Perhaps these mutations affect the ability of p53 to recruit TFIID to the promoter, leading to reduction in transcription of target genes, and increased risk to the genome integrity of a cell.

In order to investigate whether the p53 "hotspot mutants" are capable of recruiting TFIID to the DNA of target genes, the mutant p53 protein is overexpressed in sf9 insect cells and purified, and then biochemical assays and single molecule imaging experiments are performed. Co-immunoprecipitation assays determine whether there are bulk biochemical interactions between mutant p53 and TFIID, and assess how these interactions compare to those between wild type p53 and TFIID.

In the process of mutant p53 expression, baculovirus infection of sf9 cells was found to display maximum expression of p53-R175H, a "hotspot mutant," when the infection proceeds for 36 hours. After 36 hours, protein degradation was seen, which shows how toxic the p53 mutant is to the insect cells. The results of immunoprecipitation experiments show that the p53-R248Q mutant has stronger interactions with TFIID than the p53-R273H mutant. In addition, the p53-R273H mutant seems to interact with TFIID less than the wild-type p53. There does not seem to be a significant difference in interaction with TFIID, between wild-type p53 and p53-R248Q.

These results lead to the conclusion that different p53 mutants likely differ from wild-type p53 in their ability to recruit TFIID to the promoter region and transcribe p53 target genes. It would therefore be interesting to carry out further research investigating the interactions between other cancer-derived p53 proteins and the TFIID complex, and their ability to recruit TFIID to the promoter region. In addition, single-molecule colocalization techniques should be performed to study and compare the process by which wild type and mutant p53 recruit TFIID to the promoter, and to assess the length and stability of the interactions that occur during this process

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Increasing Degradation of the Huntington's Disease protein mHtt Q51 with the Proteasome Activator Blm10 purified from *Saccharomyces cerevisiae*

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Huntington's disease (HD) is a neurodegenerative disorder which causes cognitive decline affecting one out of every 10,000 Americans. HD is caused by harmful mHtt proteins expressed by the mutant Huntingtin gene (*mHTT*). Proteasomes are the central cytoplasmic and nuclear proteolytic systems and are implicated in Htt degradation. The proteasome has a modular structure consisting of a proteolytic core (CP) that is regulated by activators. We found that loss of the conserved Blm10/PA200 activators causes increased mHtt aggregation in yeast and mammalian cells. Here we seek to investigate the impact of Blm10 on the degradation of mHtt with 51 glutamines (mHttQ51) *in vitro* and hypothesize that Blm10 might increase proteasome-mediated mHttQ51 degradation. To test this we isolated Blm10-CP (BP) complexes from yeast. A degradation assay was performed with tagged HttQ51in the presence of BP, CP, or H_2O. mHtt degradation was analyzed via electrophoresis and immunoblotting. We found that the CP degrades mHtt without ATP and ubiquitin. Additionally, Blm10 significantly accelerated the degradation. Our data suggest that PA200 might impact the aggregation of mHtt in mammals.

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Knocking Out a Specific Gene in Human Cells by using the Bacterial CRISPR System

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Hantavirus infections lead to potentially fatal hemorrhagic fever in humans. Vaccines or antiviral therapies do not exist for this virus, and the biological pathways utilized for cellular entry by these viruses remain poorly characterized. Ongoing studies in the lab have implicated the EMC4 gene as a factor in hantavirus entry into human cells. EMC4 is a ER-membrane complex protein of unknown function. Recently, Clustered Regularly Interspaced Short Palindromic Repeats /CRISPR-associated system (CRISPR/Cas9) has emerged as an efficient tool to specifically knock genes out. We employed the CRISPR/Cas9 system to knockout the EMC4 gene in the human Hap1 cells and tested if this blocks the entry of Andes virus, a new world hantavirus, into these cells. Through the use of the surveyor assay and gene sequencing we have confirmed knocking out the EMC4 gene in 2 independent cell clones. Preliminary tests, however, show that EMC4 knockout in Hap1 cells does not block Andes virus glycoprotein-mediated entry.

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Functional Analysis of Homeobox Transcription Factors Six3 and Six6 in Retinal Development

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Introduction

The retina converts light entering the eye into sensory information which the brain interprets as vision. Multiple cell types of the retina differentiate from retinal progenitor cells (RPCs).[1] Homeobox transcription factor **Six3** represses the Wnt/β-catenin signaling pathway and controls the proliferation of RPCs.[2,3] Conditional deletion of Six3 ablates RPC formation.[3] Both Six3 and **Six6** are expressed in RPCs. We have recently shown that Six3/Six6 double-null mice display defective RPC maintenance, leading to a reduced diversity of cell types, smaller retinal size, and composition mostly of amacrine cells. This phenotype is not evident in either Six3 null or Six6 null mice.

We have identified an enhancer region that mediates the onset of gene expression marking photoreceptor cell commitment. We anticipate that inserting this enhancer will promote normal retinal development.

Methods

Create Six3/Six6 null mouse line using Cre/LoxP system. Verify genotype of embryos with PCR using primers designed to identify Six3 Lox P, Six3 null, Six6 WT, LacZ, and Cre. Visualize mRNA expression using in situ hybridization in whole-mount or on sections. Delineate the core enhancer and the cell lineage where the enhancer is active in transgenic mice through a series of constructs.

Results

We were able to

- Identify Six3/Six6 double mutant embryos
- Generate a cassette in which 2A sequence is placed immediately upstream of a Cre coding region and a removable Kan selection fragment. This cassette will be used for recombineering.

Future Directions

We will create transgenic mice to delineate the core enhancer.

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PDZK1 interacts with Kinesin1 to aid in trafficking of organic anion transport protein 1a1 to the plasma membrane of hepatocytes

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Abstract:

Organic anion transport proteins (OATPs) are a family of proteins that facilitate the removal of organic anions from the circulation. Previous studies from our lab indicate that oatp1a1 interacts with PDZK1. In PDZK1 knockout (KO) mice, oatp1a1 in hepatocytes is mis-localized into intracellular vesicles due to a preference for minus end motility. Co-localization studies of oatp1a1-containing mouse vesicles showed that there is significantly less plus end motor Kif5B (Kinesin 1) in KO as compared to WT vesicles. This suggests the hypothesis that PDZK1 plays a role in recruiting Kinesin 1 to oatp1a1-containing vesicles to traffic oatp1a1 to the plasma membrane. The current study investigates this hypothesis.

PDZK1 fused to a GST tag was prepared and checked for expression on SDS-PAGE. The GST/PDZK1 fusion protein and the control GST protein were then attached to GSH beads and incubated with mouse liver homogenate in 1% triton. Following incubation, the beads were washed and western blotted for the heavy chain (HC) of Kinesin 1. The pull down assay performed in this study (figure 5) indicates that Kinesin 1 may be interacting with PDZK1. However, further pull down studies are needed to confirm the interaction between PDZK1 and Kinesin 1.

Notch Signaling Regulates Heart Valve Development

Heart valve development begins at embryonic day 9.5 (E9.5) when endocardial cells undergo endocardial-to-mesenchymal transformation (EMT), invade the cardiac jelly and form cardiac cushions in the process. The cushions are subsequently remodeled into mature valve leaflets. Notch signaling in the endocardium has been shown to promote EMT. However, the role of endocardial Notch signaling in valve remodeling has not yet been established. To address this question, we generated mice models with loss of function of Notch in the valve endocardial cells by deleting Notch pathway component Notch1, Rbpj or Jagged1, and analyzed the valve morphology in these mice at E16.5 by H&E staining. We found that conditional deletion of Notch1 or Rbpj in valve endocardial cells resulted in thickened aortic valves. In contrast, conditional deletion of Jagged1 produced no detectable phenotype in aortic valve size. However, immunostaining of Jagged1 antibody indicated that the expression level of Jagged1 was comparable between wild type and mutant embryos, suggesting inadequate deletion of Jagged1. Taken together, our findings demonstrate that Notch signaling is essential for valve remodeling. However, further work is needed to determine if Jagged1 is the ligand interacting with Notch1 in the valve endocardial cells to regulate valve remodeling.

Authors: Jenna Petronglo, Yidong Wang, Bin Zhou

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Characterizing Which Domains of Myc are Required for Genomic Instability

Myc is a transcription factor that modulates the expression of up to 15% of all genes. Under normal conditions, Myc is highly regulated and is involved in numerous biological processes, including cell growth, cell proliferation, development, and apoptosis. In addition to being required for normal development, misregulation of Myc causes a large number of cancers. Myc-dependent regulation of growth and proliferation are likely to be key to Myc's oncogenic capabilities. In addition, Myc overexpression causes DNA double stranded breaks, which leads to genomic instability, a hallmark of cancer and prematurely aging cells. However, mechanisms underlying Myc-mediated genomic instability are not well defined. In this study, we are using the model organism *Drosophila* as it has a single, highly conserved Myc ortholog. Using an *in vivo lacZ* reporter transgene, our lab has previously shown that Myc overexpression increases mutation frequency and decreases lifespan. Conversely, reducing Myc decreases mutation frequency and increases lifespan. Myc levels therefore influence mutation load and lifespan. To further characterize how Myc causes genomic instability and whether this is linked to accelerated aging, we will test which domains of Myc are required for these activities. To do this, we will take advantage of existing UAS-Myc transgenes that delete specific functional domains of Myc. Myc's N-terminal box 2 (MB2) and Myc box 3 (MB3) domains are implicated in recruiting histone acetyltransferases to influence local chromatin structure and activate transcription. Myc's C-terminal basic- helix- loop- helix- zipper (BHLHZ) domains are essential for Myc to dimerise with Max and bind to the DNA sequence CACGTG (or variants thereof). By defining which domains are required, we will gain insight into how Myc increases genomic instability.

Functional Analysis of KDM5C Intellectual Disability Alleles in Drosophila

In vertebrates, there are four KDM5 paralogs: KDM5A, KDM5B, KDM5C and KDM5D. While KDM5A and KDM5B are implicated in cancer, mutations in KDM5C cause intellectual disability (ID). This project is focused on understanding the link between loss of KDM5C and ID. KDM5C-induced ID can be non-syndromic or syndromic to include phenotypes including seizures, increased aggression, speech impairment and spasticity. A total of 22 mutations in KDM5C have been linked to X-linked ID, 13 of which are missense mutations. Because the KDM5 family of proteins are multi-domain transcriptional regulators, it is likely that these mutations cause neurological phenotypes as the result of gene regulatory defects. The goal of this project is therefore to understand the transcriptional and phenotypic defects associated with ID-associated mutations using the model organism *Drosophila*. Flies have a single, essential, *kdm5* gene, and we are able to generate intellectual disability-associated alleles in the same genetic background, allowing us to systematically compare the gene expression and phenotypic consequences of disease-associated mutations. While the amino acids affected in all 13 KDM5C missense mutations are conserved in *Drosophila*, we are choosing to initially generate flies carrying the equivalent of three of these mutations: A77T, P544T and F642L. These mutations were chosen because they cause severe ID and have additional syndromic features (e.g. seizures and spasticity) that can be examined in flies. By understanding the gene expression defects and phenotypes of these alleles, the long-term goal of this project is to be able to identify novel therapeutic targets to improve the quality of life for affected patients.

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SAX-7/L1CAM acts as receptor/co-receptor for the Kallmann Syndrome protein KAL-1 in *C. elegans*

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Kallmann syndrome (KS) is a hereditary disease characterized by anosmia and infertility. *KAL1 is* the best-studied disease-causing gene and encodes an extracellular matrix associated glycoprotein, but the majority of genes causing KS remain elusive. KAL1 is conserved in *C. elegans*, allowing us to use this powerful genetic system to screen for genes that interact with *kal-1*. Misexpressing the homolog of KAL1 in *C. elegans* causes a highly penetrant axonal branching phenotype. In a forward genetic screen for modifiers of this phenotype we identified *sax-7*, which is the *C. elegans* ortholog of the L1 cell adhesion molecule (L1CAM) that when mutated leads to CRASH syndrome in humans. Genetic analysis of a stereotypical branch of the hermaphrodite-specific neuron HSN showed that *kal-1* and *sax-7* are required for the formation of this branch, thus showing their function as branching factors. Double mutant analysis of this phenotype revealed that *kal-1* and *sax-7* act in the same genetic pathway to promote the formation of this branch. Furthermore, we show that KAL-1 and SAX-7 can form a complex *in vitro*, suggesting that SAX-7/L1CAM may be acting as a receptor/co-receptor for KAL-1. Since we have previously shown that other genes that we have identified in our screen have a role in KS, L1CAM represents a new candidate gene to be mutated in still elusive cases of KS in humans.

Splicing of the CASQ2 gene in CPVT

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited rhythm disorder of the ventricles, which is characterized by dizziness, light-headedness, and syncope. CPVT can have dramatic consequences and cause sudden cardiac death. Calsequestrin-2 (*CASQ2*) and Ryanodine receptor 2 (*RYR2*) mutations have been found in CPVT patients. Mutations in *RYR2* account for ~70% cases, while mutations in *CASQ2* for 7-8%. In collaboration with Dr. McDonald, we identified a patient with a putative CPVT diagnosis. Next Generation Sequencing of this patients' germline DNA confirmed the diagnosis by detecting two mutations in *CASQ2*: a stop mutation c.199C>T and a splice site mutation c.532+1G>A. The splice site mutation has been reported once before, however the effect on splicing at the mRNA level has never been investigated. Therefore, we wanted to investigate splicing defects arising as a result of the c.532+1G>A mutation. *CASQ2* is widely expressed in the heart; however, due to the difficulties to obtain cardiac tissues we decided to investigate the expression of *CASQ2* in the platelets, a cell type that was reported as expressing *CASQ2*. We used a commercially available heart mRNA sample as a control. We also designed a set of primers to assess the purity of our platelet mRNA. Currently, we are optimizing these primers, which will allow us to proceed with determining the expression of CASQ2 in platelets. Therefore, despite our efforts we are still in the process to establish the experimental model that ultimately will allow us to determine how the c. 532+1G>A mutation affects the splicing of CASQ2.

Therapeutic strategy to treat human papillomavirus (HPV)-related cancer: Use of nanotechnology and HPV18 E6/E7 gene knockdown

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Papillomaviruses (PVs) are a diverse family of double-stranded DNA viruses that have been identified in mammals, birds and snakes. To date, over 150 distinct human papillomavirus (HPV) types have been characterized and predominantly sorted into alpha-, beta- and gamma-papillomavirus genera, the majority of which are not associated with known disease. The alpha-HPVs preferentially infect the genital tract, of which, approximately 13 types are considered as high-risk for the development of precancerous and cancerous epithelial lesions. These infections, if persistent and left untreated, can progress to malignancy. Specifically, two high-risk types, HPV16 and HPV18, are the two most prevalent high-risk HPV types responsible for 70% of cervical cancer globally. HPV16 and HPV18 are preferentially associated with squamous cell carcinomas and/or adenocarcinomas of the cervix, respectively. The HPV genome is divided into three regions: the early gene region, the late gene region, and the non-coding regulatory region (URR). Two early genes, E6 and E7, function as oncogenes. Among the high-risk types, E6 and E7 have been shown to interrupt cell cycle regulation and cell turnover, facilitating host neoplastic cell progression. E6 functions to disrupt apoptotic regulation via interactions with the tumor suppressor p53 and hTERT, the catalytic subunit of telomerase. E7 functions in the disruption of cell cycle regulation via the pRb pathway, driving the cell into DNA synthesis that disproportionately benefits the virus. High-risk HPV E6 and E7 oncogenes are capable of immortalizing cells *in vitro*.

RNA interference (RNAi) methods to silence post-transcriptional gene expression debuted during the end of the 20th century. siRNAs (short interfering-RNA) are two single-stranded RNAs typically 21-23 nucleotides (nt) long, consisting of a 19bp duplex and a 3' 2nt overhang. The antisense strand of the siRNA targets mRNA recognition, influencing its cleavage and degradation and inhibiting protein expression (McManus and Sharp 2002). In 2006, Tang *et al*., used siRNAs to identify the role of alternative splicing on the polycistronic message encoding for HPV16 and HPV18 E6 and E7. Effective knockdown in HPV16 and/or HPV18 positive cell lines confirmed the utilization of alternative splicing in HPV16 and HPV18 E6 and E7 overexpression necessary for HPV associated cellular immortalization (Tang, Tao et al. 2006). We have designed an experiment using the same siRNAs to test the efficiency of nanoparticle-mediated delivery of siRNA to knockdown HPV18 E6 and E7 in HeLa cells. HeLa cells contain integrated HPV18 DNA and are dependent on the production of E6 and E7 for survival. Nanoparticles are a new molecular tool used to deliver molecules to cells. Nanoparticles are sized between 1 and 100 nanometers and can freely diffuse across cellular barriers, including the nuclear envelope, due to their particular physical and chemical properties. The expected result is to observe effective gene knockdown of HPV18 E6 and E7 transcripts resulting from the nanoparticle-mediated delivery of the siRNAs. In addition, we expect a phenotypic result in that the cells should undergo apoptosis and die, due to the suppression of E6 and E7 expression.

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Protease Footprinting Assays of Nucleoplasmin Interaction with Histones H2A/H2B

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Histone chaperones are proteins that bind to histones to prevent undesirable interactions with free nucleic acids or proteins and deposit them onto DNA. Nucleoplasmin (Npm) is an abundant pentameric histone chaperone specific for H2A/H2B found in the *Xenopus laevis* egg. Npm has three distinct domains: the N-term flexible region; the pentamer forming hydrophobic core; and the C-term flexible tail. Npm also has three acidic patches, which are characteristic domains found in histone chaperones. Although the crystal structure of the Npm pentamer core is solved, the interaction sites between Npm and histones and how the acidic patches are involved in the interaction remain largely unknown. Furthermore, Npm contains extensive post-translational modifications (PTMs) in the egg. We and others showed that Npm phosphorylation increases its affinity for histones, but the mechanism of how PTMs affect Npm binding to histones remains yet to be elucidated. In this study, we used protease footprinting assays to show that PTMs on Npm in the egg afford higher protection of histones from digestion compared to unmodified Npm. Using Npm truncation mutants, we found that the second and longest acidic patch of Npm is critical for this extra protection and that the C-terminal most positive region weakens this protection. Together, our work shows PTMs on Npm allows the second acidic patch to bind histones possibly by disrupting the intramolecular interaction between the C-terminal most positive region and the second acidic patch.

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The Role of CPA6 in Zebrafish Seizure-like Behavior

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Carboxypeptidase A6 (CPA6) is an enzyme which binds to the extracellular matrix and cleaves C-terminal hydrophobic amino acids from proteins and peptides. Mutations in human *CPA6* have been associated recently to febrile seizures and temporal lobe epilepsy. *In vitro* studies showed these mutations had reduced CPA6 protein levels and/or activity in the extracellular matrix. Previously, knockdown of CPA6 in zebrafish demonstrated increased seizure-like activity. To further study this, zebrafish were treated with another morpholino to knock down CPA6. Behavioral assays showed a significant decrease of seizure-like activity in morpholino-injected fish, in direct contrast to the previous results. These new findings suggest that one of our morpholinos is subject to off-target effects. Additional studies with CPA6 mRNA injections yielded no significant differences in seizure-like activity from the control.

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Elevation of Circulating Microparticles in Cerebral Malaria

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Microparticles (MPs) have been shown to be associated with severe *Plasmodium falciparum* malaria. We set out to explore the association of MPs with clinical outcomes in a cohort of Malawian children with cerebral malaria (CM). MPs are derived from various cell types during activation or apoptosis and play a role in disease pathogenesis through cell signaling and immunogenic effects. Using flow cytometry to recognize cell-specific markers, we quantified the amount of MPs derived from platelets (PMPs), red blood cells (RMPs), endothelial cells (EMPs) and leukocytes (LMPs) in plasma samples from children with CM. We observed no EMPs and LMPs and a very large distribution of RMPs in the samples. We will determine if RMPs are associated with disease outcome, and if they are cleared after infection. Achieving a more complete understanding of the role of MPs in CM will lead to a better understanding of disease mechanisms, laying the foundation for more targeted disease therapies and better diagnostic tools.

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Prr7 is a Novel Regulator of the Transcription Factor, c-Jun, in Neurons

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Proline rich 7 (Prr7) is a transmembrane protein found in the postsynaptic density (PSD) of neurons in rat brains[1]. Prr7 contains a nuclear localization signal and can undergo cleavage and translocation to the nucleus (as a small fragment, deltaN). Previous work in our lab showed that NMDA (N-methyl-D-aspartate) receptor activation can trigger this translocation. This finding has cultivated the idea that Prr7 communicates synaptic events to the nucleus to regulate gene expression necessary for promoting long-term changes in synatic function. [2]Hrdinka et al., (2011) showed, that expression of Prr7 in Jurkat cells up-regulated the levels of the transcription factor, c-Jun. As of yet, it is unclear how Prr7 effects the level of c-Jun and what the exact effect is. In neurons, c-Jun levels are regulated by the F-box protein Fbw7g. Fbw7 is part of the SCF (Skp1-Cullin-F box) RING-type E3 ubiquitin ligase[3]. Interestingly, Prr7 contains a phosphodegron motif, which is recognized by Fbw7. In this study I aimed to identify how Prr7 regulates c-Jun protein levels by examining the interaction of Prr7 with Fbw7g. My data indicated that DeltaN Prr7 interacts with cJun and that deltaN Prr7 co-localizes with Fbw7g and c-Jun in HEK cells. In addition, I found that DeltaN Prr7 protects cJun from ubiquitination by Fbw7g. I also tested this in neurons and found a decrease in levels of c-Jun when Prr7 was knocked down. Together, these results suggest that Prr7 acts as a novel synapse to nucleus messenger to regulate the ubiquitination levels of the transcription factor cJun, in Neurons.

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Directed mutagenesis of HIV-1 Tat residues involved in secretion

The Tat protein catalyzes the efficient transcription of mRNA from the integrated HIV-1 genome in infected cells. Tat binds to the trans-activating response element (TAR) at the 5' ends of nascent HIV-1 RNA transcript RNA, and recruits host cell transcriptional machinery to increase the processivity of transcription.

Tat has the ability to be secreted from infected cells and to be taken up by uninfected bystander cells, where it can drive the transcription of cellular cytokine genes involved in the development of HIV dementia. It has been shown that Tat exits the cell through a non-classical secretory pathway, and two regions in the Tat protein are critical for this secretion – residues 49-RKK-51 in the Tat basic region, which associates with leaflet-bound $PI(3,4)P_2$ to localize the Tat at the plasma membrane; and W11, which inserts into the membrane and allows Tat to exit. To better understand the role of these residues in the Tat protein secretion, we used site-directed mutagenesis to create a series of mutants in a Tat genes cloned into a mammalian expression vector. The resulting effects of the mutations on Tat protein egress was then measured using a transcellular transactivation assay, in which HeLa cells are transfected with Tat-expression constructs, and then cultivated with either TZMbl or HLM-1reporter cells. If the Tat is able to be released from the producer cells and taken up by the reporter cells, reporter cells would generate a luciferase signal (TZMbl cells), or produce infectious HIV detectable with p24 Elisa assay (HLM0-1 cells). We predict the Tat W11 and 49-RKK-51 mutants to be deficient in exocytosis and unable to drive a detectable reporter cell response.

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Construction of a D29 fluorophage for the analysis of *Mycobacterium leprae*

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Leprosy, an age-old scourge, remains a persistent problem in certain areas of the Developing World. The causative agent of leprosy, *Mycobacterium leprae*, has never been successfully grown on artificial cell culture media. Sources of *M. leprae* are limited to tissues from infected humans, nine-banded armadillos, or mouse footpads. The inability to culture *M. leprae* presents a major impediment to the visualization of metabolically active leprosy bacilli, as well as to the study of this pathogen's physiology and genetics. Of the 524 fully sequenced and annotated mycobacterium-specific phages, D29 is the only one known to infect *M. leprae*. D29, therefore, presents an exciting opportunity to revolutionize the study of *M. leprae*. To create a new *M. leprae* specific fluorophage, a cosmid containing the reporter gene *mVenus* under the control of the highly efficient phage L5 promoter (pYUB1551) was cloned into a D29 phasmid backbone, yielding D29-1551. The resulting shuttle phasmid was amplified in *E. coli* and electroporated into the permissive fast-growing host, *Mycobacterium smegmatis*. The generation of a high-titer lysate is currently in progress. For this construct to effectively enhance visualization of metabolically active *M. leprae*, the cells must be intact. Due to the virulent nature of D29, recombineering will be used to delete holin and endolysin proteins which are responsible for bursting the mycobacterial cell wall during lytic phage infection. This work will also lead to the first *in vitro* drug susceptibility testing of *M. leprae*.

Absolute per cell quantification of crystallin mRNA in the developing mouse lens

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The developing mammalian lens is an advantageous model system for the study of many genetic and epigenetic regulatory mechanisms. Lens cells undergo characteristic changes in cellular organization, morphology and protein composition to form the mature lens which is comprised mostly of highly elongated lens fibers. These terminally differentiated and organelle-free lens fibers represent the bulk of the lens which is covered by a sheet of epithelial cells at the anterior. Lens fiber cells are principally responsible for lens transparency and refractive index and accumulate high levels of various crystallin proteins, which are expressed in a spatially and temporally specific manner during embryonic and postnatal development. For these reasons, lens development is a good system for the study of processes fundamental to organogenesis such as tissue-restricted gene expression, lineage-specification, cellular proliferation, differentiation and signal transduction, migration and cell death. We hypothesize that crystallins are expressed at very high levels per each cell, as measured by their mRNA copy number. To accommodate this high level of expression, we also hypothesize that αA-crystallin loci interact spatially with other genes forming the 3D-transcriptional hubs where multiple highly expressed genes share transcriptional machinery to optimize their expression. To effectively use the embryonic lens as a model system it is necessary to have precise quantitative data on crystallin expression throughout development. In this project we aimed to evaluate the developmental expression pattern of 10 highly expressed crystallins including □A, □B, □A1, □A4, □B1, □B2, □A, □ B, □ C, and □ D by performing an absolute quantification of the associated mRNAs at progressing embryonic stages (E14.5, E15.5, E16.5, E17.5 and P1). The data obtained will be useful for future work using crystallin expression as a model for genetic and epigenetic regulatory mechanisms.

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Identifying CRISPR/Cas9 induced mutations disrupting zebrafish *kif5Ba* and *kif5Bb*

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Highly polarized cells, such as neurons and oocytes rely on intracellular transport of diverse cargos including organelles, vesicles, proteins, and RNAs to establish and maintain their polarity, function, and viability. Kinesin-1 is an important plus-end directed microtubule used in the transport of organelles, vesicles, proteins, and RNA. Kif5 proteins homo- and heterodimerize to form the motor domain of Kinesin-1. While humans possess three *kif5*s, *kif5A*, *kif5B*, and *kif5C*, zebrafish possess five: *kif5Aa*, *kif5Ab*, *kif5Ba*, *kif5Bb*, and *kif5C*. *Kif5A* and *kif5C* are expressed neuronally and have essential roles in motor and sensory function. In contrast *kif5B* is expressed ubiquitously. Early embryonic lethality of Kif5B mouse knockouts indicates that Kif5B is essential for development; however, its precise contributions to early embryogenesis have not been elucidated. Due to partial duplication of the zebrafish genome, zebrafish possess 2 *kif5B* genes. For other duplicated zebrafish genes the functions of the single mammalian gene has been split or are partially redundant between the duplicated genes. Thus, studies of zebrafish *kif5B* individual and compound mutants are expected to provide insight into *kif5B* functions. In order to understand their roles in early development, targeted mutations were induced in zebrafish *kif5Ba* and *kif5Bb* genes using the CRISPR/CAS 9 system. This system utilizes a guide RNA, composed of a sequence complementary to target genes, and RNA that encodes the endonuclease Cas9. When microinjected into one-cell staged zebrafish embryos cells that take up both the guide RNA and the Cas9 RNA have the potential to cause mutations in the targeted genes. In order to create stable mutant lines, I screened F0 adults that were raised from CRISPR/Cas9 injected embryos for germline transmission of *kif5Ba* and *kif5Bb* mutations. Prospective founders (adults carrying mutant *kif5B* alleles) were outcrossed, genomic DNA was extracted from individual progeny, and mutations were identified by simple PCR and restriction digest based methods and confirmed by sequence analysis. Four *kif5Ba* and five *kif5Bb* founders bearing mutant alleles were identified. The identified mutations were all deletions, but varied in length ranging from 5-11bp in length. Upon sequencing, two of the *kif5Ba* and three of the *kif5Bb* mutations cause premature stop codons (nonsense mutations) while two *kif5Ba* mutations lead to deletion of two amino acids (missense mutations). Progeny from founder outcrosses (F1) are being raised in order to examine *kif5B* mutant phenotypes in the F2 progeny. Studies of individual and compound mutants will provide insight into Kif5B mediated processes and the extent to which individual *kif5s* overlap in terms of function and cargos transported.

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The Effects of HIV-1 Protease Aptamers on Virus Production

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Aptamers, oligonucleotide ligands identified by SELEX, can be used to modify or inhibit the wild type function of a protein target. Since they generally have high affinity and specificity for their targets, are non-immunogenic, and easily made by chemical synthesis, aptamers are good candidates for therapeutics. Several aptamers have been designed to inhibit the HIV-1 proteins for the purpose of developing novel anti-retroviral drugs. In earlier work from Prasad laboratory, RNA aptamers to HIV-1 protease (PR) were identified via SELEX, and were tested in binding assays to determine their binding affinity. Here, we have tested the effects of these aptamers on virus production in 293T cells. Cells were co-transfected with pNL4.3-Luc reporter HIV-1 plasmid (sub-type B), the VSV-G envelope gene, and plasmids that were designed to express each of six different PR aptamers. Virus release into the supernatant was detected by p24 ELISA. The anti-PR aptamers showed significant reduction of virus production compared to a non-transfected control, indicating that inhibiting the function of viral PR can decrease the production of virus.

Cloning and Protein Expression of the Pore Domain of the KCNQ1 Potassium Channel with GST Expression Vector

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Potassium (K^+) channels are largely involved in the maintenance of cell resting potential and the formation of action potentials. In the heart, the membrane protein KCNQ1 interacts with the β-subunit KCNE1 and is responsible for the delayed rectifier current I_{Ks}, which is involved in ending cardiac action potentials. Mutations in KCNQ1 can lead to malfunction of the channel causing Long QT syndrome (LQTS1), which manifests as an increase in the time between the beginning of the QRS complex and the end of the T-wave in an electrocardiogram. Patients with LQTS1 are more susceptible to torsade de pointes arrhythmias and sudden cardiac death. For this reason, it is important to determine the structure of the membrane protein to increase knowledge and aid future endeavors in treatment. Previously, our group cloned the pore domain portion of the human and several homolog genes using the MBP expression vector, and purification strategies are underway. The experiments in this presentation aim to explore alternate approaches to expression and purification for future structural analyses. Fusions with GST tags were successfully cloned and minimal expression was observed, but expression levels are not yet high enough for scaling up. In the future, the lab intends to attempt other techniques to purify the MBP fusions and adjust the expression protocol to increase expression of the GST fusions.

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Genetic sequence analysis of *S. aureus* enterotoxin, SEK

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Staphylococcus aureus (S. aureus), an important human pathogen, produces a wide range of proteins that contribute to its virulence and pathogenesis. One group of these protein toxins is known as the staphylococcal enterotoxins (SEs). Most SEs exhibit superantigenic properties including the ability to stimulate a large fraction of T-cells by simultaneously binding to the outside surface of MHC-II on antigen presenting cells and T-cell receptors. Activation of these T-cells leads to an exuberant pro-inflammatory cytokine response that is toxic to the host. A newly discovered SE, Staphylococcal Enterotoxin K (SEK), has been shown to be superantigenic *in*-vitro, similarly to other SEs. Unlike most SEs, however, the SEK gene is very common amongst our collection of 200 clinical isolates from New York hospitals. My project is focused on analyzing the genetic sequence of SEK, which has been shown to vary between different strains of *S. aureus*. To do this, genomic DNA was isolated from 3 different clonal groups: Methicillin-susceptible *S. aureus* (MSSA), Methicillin-resistant *S. aureus* (MRSA), and the Community-acquired MRSA clone USA300. The coding regions were amplified via polymerase chain reaction (PCR), sequenced, and then comparatively analyzed. Our findings suggest that the SEK gene is highly conserved only within the USA300 group. This finding has opened new investigations into the possible association between SEK alleles and SEK production, as well as the distribution of SEK alleles among clinical *S. aureus* isolates.

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Mutations in neuropeptide formation and release do not have an effect on synapse formation in *C. elegans*

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Abstract

C. elegans has been widely utilized in scientific study for its simple nervous system and ease of genetic analysis (Brenner 1974). Among the various interests *C. elegans* is used to explore, we were concerned with the nature of synaptic development in the B-type ray neurons. Synapses and, further, connections between neurons in a nervous system of any animal are responsible for relaying information to other cells within a system. It is for this reason paramount to study the development of these connections to better our understanding of the nervous system's form of communication. This study investigated the effects of mutations in two genes, *egl-21* and *unc-31*, in comparison to our reporter strain that contains a *pkd-2*::*rab-3*::mCherry transgene, allowing us to view both the synapses—manifested as a "synaptic ring"—in addition to the neurons. Because the *egl-21* and *unc-31* genes are required for neuropeptide formation and release respectively, we investigated whether a mutation in these genes has an effect on the development of synapses. Although the scoring data shows some alteration each mutation had on the synaptic ring in comparison to the wildtype strain, overall the gene mutations did not cause a significant deficiency in synaptic development. We conclude that neuropeptides are not required for formation of the synapses visualized by this reporter.

Labeling Glycans with Click Chemistry Tags Enables Live-Cell Microscopy

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Click chemistry involves the rapid, stereospecific attachment of small molecular pieces to form larger aggregates using reactions that are thermodynamically favorable, physiologically stable and stereospecific. Recently, the term bioorthogonal chemistry has been introduced to refer to reactions performed in live-cell environments that do not interfere with native pathways. Using bioorthogonal click chemistry with azide and alkyne groups ("tags"), we can visualize the incorporation of two tagged sugars, Ac_4ManNAl and Ac_4GalNAz, to study the formation of N-linked and O-linked glycans, respectively.

We have visualized sialylated N-linked glycans in a type of mammary tumor cell using a Cu(I)-catalyzed cycloaddition reaction to link the alkyne-tagged Ac_4ManNAl with 647-Azide. The tagged sugar, Ac_4ManNAl, is introduced into the cell culture medium and, consistent with the principles of bioorthogonal chemistry, the sugar hijacks the cell's native pathways to be taken up by the cell and processed by native enzymes to form sialylated N-linked glycans, which are expressed on the cell's surface. The 647-Azide is then introduced with the copper catalyst and covalently links to the alkyne tags of the sialylated N-linked glycans. We have visualized the dynamic diffusion of these fluorescent glycans on the cell surface and using photobleaching methods, we have quantified the existence of a mobile pool of glycans.

To verify the specificity of our bioorthogonal approach, we have begun a western blot analysis on cell lysates. Expressed Ac_4GalNAz is linked to a biotin-alkyne, and after cells are lysed, an anti-biotin antibody is used to bind the biotin-glycan complexes in the cell lysate.

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Impact of Cited2 Gene Knockdown on Muscle Development

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Muscular dystrophies (MD) encompass a range of genetic diseases that cause the degeneration of muscle tissue and the weakening of muscles. MD is known to weaken body systems and reduce life expectancy. The degenerative capacity of different types of muscular dystrophy diseases, such as Duchenne MD and Becker MD, lead many to investigate the restorative possibilities for those affected by these medical conditions. Cited-family proteins have been identified as a necessary and important component for the normal maintenance of muscle tissue. Cited2 protein (cAMP-response element-binding protein (CBP)/p300-interacting transactivator with glutamic acid (E)-aspartic acid (D)-rich tail 2) is a transcription cofactor, is expressed in fast muscle precursors and its absence leads to muscle dystrophy. In this project, we have found that the associations between *cited2* and other muscle genes that are vital during muscle development of *Danio rerio*, the zebrafish, could potentially unveil new paths for MD treatment. Through investigation of *cited2* gene, we found Cited2 to be an interacting partner with tnnt3b, myofiber differentiation delays are seen in the absence of Cited2, and muscular atrophy was seen in the absence of Cited2. Future work involves carrying out mechanistic studies of interacting partners involved in development.

Stress Management Intervention for Living with Epilepsy (SMILE): Methodology, Recruitment, and Compliance

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Many patients with intractable epilepsy continue to suffer from the debilitating implications of poorly controlled seizures, even in light of all the recent advances in pharmacotherapy. Targeting and placating the common underlying triggers that provoke seizures is essential in order to minimize seizure frequency. Studies have shown that stress is a common trigger for patients with refractory epilepsy. In order to examine the effects of stress reduction on seizure quantity in such patients, we enrolled potential study candidates into a randomized, controlled, double blind clinical trial of progressive muscle relaxation: a stress reduction technique that incorporates the successive tensing and relaxing of the major skeletal muscle groups. As such, we believe it may lessen seizure frequency when used as an add-on therapy. In conjunction with the PMR, patients completed daily diary entries via a Smartphone device, wherein they reported their mood and stress level, and documented any occurrence of seizures. The results at the completion of the clinical trial will not only offer insight as to the efficacy of our study design, but they will also have novel implications for future behavioral research, specifically as they concern maximizing seizure control in patients with medically intractable epilepsy.

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Identification of Proteins Interacting with a Negative Feedback Regulator of Macrophage and Osteoclast Development

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Proline serine threonine-rich phosphatase interacting protein 2 (PSTPIP2) is a Pombe Cdc15 Homology (PCH) protein that has been shown to play an important role in actin bundling and manipulating the cytoskeleton of macrophages. PSTPIP2 directly interacts with actin, plays an anti-inflammatory role in macrophages and is responsible for decreasing colony stimulating factor-1 (CSF-1)-induced membrane ruffling but increasing filopodia formation, motility, and CSF-1-stimulated chemotaxis. The goal of this project was to discover protein interactors of PSTPIP2 with the use of a yeast two-hybrid (Y2H) system. We confirmed over 20 proteins that are likely to bind with PSTPIP2 through this process and further investigated the possible binding sites for some of the proteins as well as the phosphorylation effects on binding. It appears that most proteins seem to bind nearer to the carboxy terminal end of PSTPIP2 and that Src induced phosphorylation negatively impacts binding with some of them. Additionally, we discovered that disease-associated mutations seen in Lupo and firewalker mice increased PSTPIP2 binding to Clic1 and had no effect on the binding for the other proteins tested.

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Distinguishing change detection from stimulus specific adaptation

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Mismatch negativity (MMN) is elicited when there is a change in the auditory environment. However, there is controversy as to whether this MMN is caused by change detection or stimulus specific adaptation (SSA). Using a repeating standard pattern of tones (XXXO) instead of a single tone (as seen in previous oddball paradigms), our study distinguished between the contributions of SSA and change detection. During the auditory task, two deviants were presented: the short deviant XO (D1) would elicit MMN to both change detection and SSA, while the long deviant XXXXXO (D2) would demonstrate change detection alone on the 4th X-tone. Participants were instructed to detect changes from the standard pattern (active condition) or watch a silent movie (passive condition). Results in the active condition demonstrated faster responses for D1 than D2, with an approximately equivalent hit rate. Significant MMN was elicited by D2 during active and passive conditions, providing evidence for change detection based MMN. Larger MMN amplitude for D1 than D2 suggests a combined contribution of SSA and change detection. This study demonstrates that MMN can reflect change detection alone. This research was supported by the National Institute of Deafness and other Communications Disorders and by the Army Research Office.

Synthetic antibody technology for structural characterization of antibody-antigen interactions

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High-throughput screening of phage-displayed synthetic antibody libraries can be used for investigating antibody-antigen interactions and is an efficient alternative to hybridoma technology for generating novel monoclonal antibodies. Synthetic antibody repertoires contain artificially diversified antigen-binding sites encoded by designed, synthetic oligonucleotides. This approach does not require immunization of a host animal and therefore has distinct advantages over traditional techniques. Using synthetic antibodies to decipher significant molecular interactions at the binding site, we aim to characterize the activation mechanisms of the BCL-2 Associated X (BAX) protein, an important apoptosis-regulating protein in the BCL-2 family. After rounds of selection, we identified a group of high-affinity synthetic antibodies for BAX. The antigen-binding fragments of these antibodies were expressed and purified for further characterization such as affinity measurement, co-crystallization, and bioactivity studies. The results show that our antibodies bind with nanomolar affinity to BAX and some inhibit BAX activation by apoptotic activator tBID. We also started investigating the molecular interaction details between Human immunodeficiency virus type 1 surface glycoprotein (gp) 120 and human monoclonal antibody (mAb) 2G12. 2G12 is one of the few available broadly neutralizing mAbs that recognizes glycans on gp120. Synthetic libraries were designed by shotgun scanning combinatorial mutagenesis to highlight residues involved in antigen binding through phage display. We are currently screening a homolog-scan library and synthesizing an alanine-scan library. In addition to mapping a comprehensive structural binding interface to understand the function of distinct side chains, we will apply new 2G12-based libraries for targeting glycan structures in other disease-related agents.

Treatment of GM2 Gangliosidosis through neuronal uptake of Hexosaminidase in an active hybrid form.

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Lysosomal storage disorders (LSDs) are inherited diseases that result from defects in neuronal lysosomal system proteins and often lead to cognitive, motor and sensory deficits, and premature death. To date, neuropathologic LSDs remain incurable. Enzyme Replacement Therapy (ERT) involves replacement of the deficient soluble enzyme through endocytosis of exogenous normal enzyme. However, the blood brain barrier (BBB) as well as poor neuronal constitutive endocytosis hinders the effectiveness of this and related approaches for CNS treatment. The goal is to combat these challenges by developing modifications to enzymes that still maintain activity and yield a form that can be readily taken up by neuronal cells. The lab has developed several peptides, based on the structure of atoxic C fragment (Hc) of tetanus toxin (TT), that show promise in both circumventing the BBB and enhancing neuronal uptake.

In the current project, two recombinant constructs of β-hexosaminidase (Hex), the enzyme deficient in Tay Sachs and Sandhoff diseases, fused with an Hc-based peptide (PEP1) via two different linkers, FLEX1 and FHF, were tested for uptake in neocortical cultures from a mouse model of Sandhoff disease. Microglial Sandhoff cells were virally infected with this recombinant DNA and subsequently confirmed positive for Hex expression and for secretion of Hex activity by biochemical assay. Secreted enzyme was concentrated to >25,000U (ng substrate cleaved at 37°/hr)/ml. Neocortical cultures from a Sandhoff disease were dosed with a fixed amount of these hybrid constructs as well as recombinant native Hex. Based on histochemical staining using a specific substrate (XHEX) for Hex activity, it was confirmed with native Hex that astrocytes and microglia take up enzymes more readily than do neurons, which were XHEX negative. The modified constructs did not produce a perceptible difference in neuronal uptake. This lack of detection may have been due to an altered ratio of active Hex A and B isozymes. Indeed biochemical assays revealed HexA-specific activity to be proportionally higher for the modified Hex versions (MUGS/MUG substrate = 0.13 native Hex; 0.18 Flex1; 0.25 FHF) suggesting these products would be less detectable by XHEX. A second uptake experiment was conducted using dosing scheme to partly compensate for this. While there was some indication of improved uptake via FHF modification, the results remain inconclusive in part due to loss of neurons from high albumin concentrations present in the current preparations.

In parallel, in an attempt to maximize neuronal enzyme uptake we created a new modified version of Hex inserted into a plasmid denoted as naldini-hexflex3-HcPEP1. This FLEX3 linker, which was digested out of a pIRES plasmid, differs from the previous linkers in being substantially shorter and less rigid. After recombinant modification, confirmatory restriction and matching of the desired sequence, the construct was nucleofected into microglial cells. Results with XHEX showed an expected 1% positive cells based on the size of the construct and confirmed production of an active hybrid enzyme. The construct is currently being packaged into lentiviral particles to be used to infect microglia cells with the construct to produce purified enzyme with which to dose neuronal cells. Further evaluation of Hex A and B content as well as further purification will be done to conduct additional tests on the Sandhoff brain cell cultures. If improved levels of uptake are observed, we may be one step closer to overcoming the obstacles of the CNS to find a cure for LSDs.

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