2011 SUMMER UNDERGRADUATE RESEARCH PROGRAM

Abstract Book

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OF YESHIVA UNIVERSITY

GRADUATE PROGRAMS IN THE BIOMEDICAL SCIENCES

Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Division of Biomedical Sciences

SUMMER UNDERGRADUATE RESEARCH PROGRAM

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Associate Dean for Graduate Studies
Director, Summer Undergraduate Research Program

2011 Summer Undergraduate Research Program

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The Effects of Different Forms of Radiation on Energy Production by Melanized Cells

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Melanin is a pigment produced by the cell wall of *Cryptococcus neoformans*; this occurs when a substrate is available. This pigment protects the cell from many environmental hazards, including radiation. One purpose of this pigment could possibly be to convert harmful forms of radiation into heat which could also possibly promote growth of the melanized cells by triggering a higher state of excitement for the electrons involved with the transfer of NAD^+/NADH (Nicotinamide Adenine Dinucleotide) inside of the cell and cause a decrease in ATP (Adenosine Triphosphate). *C. neoformans* was used as the subject of the research. Melanized and non-melanized samples were used to see if ultraviolet rays, light, or gamma radiation would cause adverse or favorable conditions for the *C. neoformans* cells. It was hypothesized beforehand that melanized cells would show a higher level of activity within these conditions proving it to be favorable. Standard conditions were done for both samples to observe the cells under normal conditions. As well, XTT and MTT assays were performed to see the level of activity within the cells by detecting the level of reduced tetrazolium salts by the optical density of the supernatant with a spectrophotometer. From the results that were observed, it can be concluded that melanin alters the growth of cells when affected by radiation. With these findings a mechanism could be unraveled to possibly provide a source of energy harvest from melanin.

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The relationship between colony size and lifespan in *Caenorhabditis elegans.*

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The interplay between genes and the environment has revealed the plasticity of aging and the possibility of lifespan extension. While the influence of diet on lifespan has been extensively studied in invertebrates, recent work suggests that the social environment may also affect invertebrate longevity. Colony size is often manipulated to determine the influence of different social environments. A recent study showed that honeybees live longer in smaller colonies than larger ones. Similarly, Zur et al. (2009) showed that solitary Tephritidae fruit flies consumed less and lived longer than those in pairs.

Despite the frequent use of *C. elegans* in lifespan analysis, the relationship between its social context and lifespan remains to be established. While the wild-type laboratory strain is considered solitary, previous studies suggest that conspecifics can have significant effects on one another such as normal growth size and mechanosensory response. Furthermore, previous longevity studies in *C. elegans* lack a fixed colony size with a range from 5 to 30 worms per plate. Thus, the possible influence of colony size on lifespan analysis warrants further examination.

The present study investigated the effects of colony size on the lifespan of *C. elegans.* We observed 90 isolated worms and 90 colony-reared worms on 3 plates (30/plate). Based on previous experiments, we hypothesized that the isolated worms would have a longer lifespan than colony-reared worms.

Our results did not show a significant difference between the lifespan of isolated and colony-reared worms. These results do not correlate with previous studies, suggesting that previously seen conspecific effects may not be due to nematodes' physical presence. However, this research is in its early stages as we continue to investigate the influence of social context on aging in various conditions.

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A Unique Presentation of Atypical Hemolytic Uremic Syndrome

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Atypical hemolytic-uremic syndrome (aHUS) is a rare, often recurrent disease characterized by hemolytic anemia, thrombocytopenia and acute renal injury. Half of patients diagnosed with aHUS develop end stage renal disease with 25% mortality. Atypical HUS is not easily recognized and because of its rarity, there is a lack of clinical experience within the medical community pertaining to early diagnosis and treatment.

We present the case of an 8 year old female with no prior medical history, who presented with aHUS. The patient presented to the Montefiore ED with low hemoglobin and hematocrit, a low platelet count and elevated blood urea nitrogen (BUN), uric acid and creatinine. Initial concern was for both typical and atypical HUS, malignancy and thrombotic thrombocytopenia purpura (TTP). Evidence of normal bone marrow function, negative blood cultures and an assay demonstrating normal ADAMTS13 activity rendered the diagnoses of typical HUS, TTP and malignancy less likely. The patient was initially managed with supportive care. Her renal function however, continued to worsen. Suspicion of aHUS, rarely seen in patients over 5 years of age, was heightened and the decision was made to initiate plasmapheresis. Following repeated sessions of plasmatherapy, the patient's hemoglobin, hematocrit, BUN and creatinine levels as well as platelet count returned to normal. The patient is continuing plasmapheresis while the results of genetic testing are pending.

We use this case to highlight the presentation of a rare disease and raise management issues related to promising, potential new treatments.

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Identification of Novel Type 1 Diabetes Autoantigens in NOD Mice

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Type 1 diabetes is an autoimmune disease in which the body reacts to and destroys its own beta cells via an immune response. These beta cells are located within pancreatic islets. Autoreactive CD8 T cells target autoantigens, or antigens synthesized by the body, within the beta cells and thus infiltrate the islets. Once they have infiltrated, the CD8 T cells destroy the beta cells. Low levels of insulin caused by the loss of beta cells triggers the onset of diabetes.

The present investigation seeks to identify new autoantigenic proteins/protein fragments for future study. We previously developed a list of genes that code for potential autoantigens using data from bioinformatics searches and a new algorithm, which takes into account the gene's expression level and specificity in the islet as a whole and in the MIN6 beta cell line. To obtain CD8 T cells for our antigen screens, pancreases from NOD (non-obese diabetic) mice are harvested and digested. Then the islets are isolated and cultured to allow the T cells to migrate out of the islets. T cells are collected and mixed with antigen presenting cells (APCs) and potential autoantigens in an ELISPOT plate. The ELISPOT plate will show evidence of an immune reaction in the form of colored purple spots. The number of spots is then read by an automated ELISPOT plate reader. Prior to testing the potential autoantigens, we used NRP-V7 (a known mimotope of the IGRP autoantigen) to test the efficiency of our two potential APCs—RMA-S/K^d and T2/K^d.

Thus far, we have concluded that T2/K^d is the better APC to use for further experimentation. We have also identified autoantigenic peptide fragments derived from several proteins, including chromogranin A and glucagon. Chromogranin A is a known CD4 T cell antigen, but this is the first identification of chromogranin A peptides as CD8 epitopes. The identification of glucagon-derived peptides as CD8 T cell antigens is also of considerable interest. It may suggest that, in addition to beta cells, glucagon-producing alpha cells may also be targets of the autoimmune attack as type 1 diabetes progresses.

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Characterization of Mice Receiving Growth-Arrest-Specific Protein6 (gas6) during MOG-induced Experimental Autoimmune Encephalomyelitis (EAE), a mouse model of inflammatory disease.

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Growth arrest-specific protein6 (gas6) is a growth factor expressed and secreted by neurons. Our research has shown that gas6 is important for oligodendrocyte survival and that dysregulation of protective Gas6/receptor signaling occurs in MS lesions. In this study, we evaluated the beneficial effect of gas6 during experimental autoimmune encephalomyelitis (EAE). C5BL6 mice received a tail vein injection of gas6-AAV9 and/or green fluorescent protein (GFP)-AAV9, in a 200 µl maximum volume, prior to or following EAE sensitization. The average clinical scores of the gas6-AAV9 treated mice were not significantly different from those receiving saline. In one experiment we did observe a significant difference between the groups receiving gas6-AAV9 + GFP-AAV9 and the saline-treated mice. RNA was isolated from all mice treated, and quantitative RT-PCR (qRT-PCR) assays were conducted with gas6, GFP and GAPDH primers. No GFP was detected in the CNS or liver in mice treated with GFP-AAV9 36 or 45 days post EAE induction. Mice injected with 2x10e11 gas6-AAV9 expressed less gas6 in their CNS compared to the amount expressed in the saline-treated and naive mice. Evaluation of the RNA integrity by a Bioanalyzer assay determined that the RNA was of high quality. Therefore the lack of detect-ability of GFP and gas6 transcripts was not due to RNA degradation. Our results confirmed a recent report that determined that at least 10e12 GC of AAV9 is required to successfully transduce the CNS. Since the maximum volume that can be injected in mice has a concentration of 2x10e11 GC, the current AAV9 lentivirus is not an effective method of upregulating gas6 in the CNS. Future studies will work on introducing gas6 by the cannula directly into the CNS of mice sensitized to EAE.

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The Effects of CSF-1R Mutation on Macrophage Podsome Formation and Function

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Macrophages are critical to normal immune function and tissue development. However, a paracrine loop between carcinoma cells and macrophages has been reported and shown to promote tumor cell invasion and metastasis. As such, it is important to understand the signaling mechanisms which control macrophage-carcinoma cell communication and motility.

The aforementioned paracrine interaction involves secretion of EGF from macrophages and CSF-1 from carcinoma cells. CSF-1 is the major regulator of tissue macrophage differentiation, proliferation, and motility and regulates podosome assembly via PI 3-kinase. CSF-1 binds to the CSF-1 receptor tyrosine kinase (CSF-1R) and induces phosphorylation of seven tyrosine residues. Using CSF-1R$^{-/-}$ bone marrow macrophages rescued with specific CSF-1R tyrosine-to-phenylalanine mutations we have examined the role of CSF-1R tyrosine phosphorylation in podosome assembly and matrix degradation when cultured alone or co-cultures with carcinoma cells.

Our results indicate PI-3 kinase as an important regulatory element in podosome formation. In the absence of a direct pathway to PI-3 kinase activation, macrophages showed a decrease in podosome formation following continuous exposure to CSF-1 on glass. This trend was not evident when the cells were plated on fibronectin highlighting the difference between these surfaces and raising questions as to whether podosome formation is regulated by mechanisms dependant on the cell's extracellular environment. In co-culture most of the macrophage cell lines showed an increase in podosome formation and matrix degradation compared with the mono cultures of those lines, but again mutants defective in PI3-kinase binding showed the opposite trend, highlighting the importance of PI-3 kinase.

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The Study of Differential Methylation in Autism Spectrum Disorders (ASD) and Hepatocellular Carcinoma (HCC) and of Covert Mosaicism in ASD

By

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Our lab is currently researching epigenetic phenomena in two human diseases: hepatocellular carcinoma (HCC) and autism spectrum disorder (ASD). It has already been established that there are epigenetic differences between hepatocellular carcinoma and healthy hepatocytes. Our hypothesis is that the Hepatitis C Virus induces epigenetic changes in hepatocytes that represent an intermediate step in the progression to the cancer epigenome. We are currently employing bisulfite massarray to validate differential methylation sites in HCC samples discovered from HELP-tagging assays.

There is less known about the epigenetic dysregulation in autism since it is difficult to get samples from the affected organ, the brain. We have, therefore, decided to use the embryologically related buccal epithelial cells, which like neuronal cells are of ectodermal origin, to evaluate the genetic content and epigenome of children with and without ASD born to mothers over 35. We are particularly interested in the effect of maternal age on the development of ASD. We hypothesize that mechanisms involved in pathology of the aging germline may contribute to the etiology of ASDs. Not only is there evidence that epigenetic regulation can decay with age, but a mother's oocyte may undergo meiotic I non-disjunction before zygote formation because of its arrest in prophase I. Meiotic I non-disjunction can ultimately generate an embryo with mosaic cell lines with covert aneuploidy and uniparental disomy. We are currently processing DNA from subjects to perform quantitative single nucleotide polymorphism (SNP) genotyping to identify covert mosaicism and HELP-tagging assays to study epigenomic dysregulation.

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Do Ribosomal defects cause cell competition through the Salvador-Warts-Hippo Tumor Suppressor Pathway?

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Cell competition can occur in mosaic tissues consisting of two, otherwise viable, populations of cells. One cell type ("winners") proliferates at the expense of the other ("losers"). Cell competition can occur in a number of different contexts, including interactions between wild type cells and Minutes – cells which are heterozygous for certain ribosomal proteins mutations – where wild type cells become winners, expand in the tissue, and induce apoptosis within the loser cell population. However, the mechanism by which a cell identifies itself to become a winner or a loser is poorly understood. Interestingly, antibody staining have shown that minute cells express a higher level of the Fat, a cadherin that spans the apical membrane of epithelial cells, than wild type cells in both competitive and non-competitive environments. An important function of FAT is helping activate the Salavador-Warts-Hippo (SWH) tumor suppressor pathway. This observation led us to wonder whether relative levels of fat protein might determine winner and loser cell identity during cell competition. We set out to determine whether these relative differences in fat levels are controlled at the transcriptional or at the protein level, and if any other members of the SWH pathway were elevated. We isolated total RNA from wing imaginal discs of Minute (67C) animals and their corresponding wild type controls. Quantitative RT-PCR revealed that fat and other members of the SWH pathway are transcriptionally elevated. Future directions include examining other minutes, and investigating possible relationships between relative differences in fat and cell competition.

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Elucidating the Regulation of Myosin-IIA Heavy-Chain Phosphorylation by GPCRs

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Cancer becomes truly dangerous at the point of metastasis – when cancer cells migrate to distant portions of the body and generate new colonies. Chemotaxis, the migration of cells in response to chemical stimuli, is thought to play a significant role in metastasis. Cytoskeletal rearrangement after stimulation has been somewhat characterized; the main elements are protrusion of a lamellipodium at the anterior end of the cell and retraction of the posterior end. Nevertheless, the underlying mechanisms are not well understood. It is known, however, that contractile forces are necessary for this activity, and type II myosin supplies force by pulling on f-actin stress fibers. It has been demonstrated that when a cell is stimulated by EGF, the heavy chain of nonmuscle myosin-IIA (MIIA-HC) is transiently phosphorylated at S1943 in a PI3K-dependent manner (as evidenced by lack of phosphorylation in the presence of the PI3K inhibitor wortmannin). This phosphorylation inhibits myosin-IIA filament assembly, allowing breakdown of myosin-IIA filaments and movement of myosin-IIA monomers toward new adhesion sites. We used MDA-MB-231 carcinoma cells to test for similar phosphorylation in response to LPA, which signals through GPCRs. Cells were quiesced, stimulated with 10μM LPA, and lysed at time points between 0 and 10 minutes. Levels of total and phosphorylated myosin were examined by Western blotting and compared to determine phosphorylation at each time point. A transient increase in phosphorylated MIIA-HC was observed, although further repetitions will be needed to verify statistical significance. Once this phenomenon is confirmed, the role of PI3Ks in LPA-induced MIIA phosphorylation can be examined by blocking production of PI3Ks with shRNA.

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TRIM5α Protein Binds HIV-1 Capsid in vitro

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HIV-1 assembles into a complex consisting of a spherical, Gag polyprotein shell (capsid-CA) and a concentric, conical Gag polyprotein core (nucleocapsid-NC) that houses the viral RNA. Gag protein mutations that interfere with the formation of the core 4result in inhibition of infection, thus the conical core and capsid complex is needed for infectivity. The cytoplasmic protein TRIM5α (Tripartite motif-containing protein 5) has been shown to block HIV-1 infection in Old World monkeys by interfering with the uncoating mechanism. The purpose of this project is to produce synthetic CA-NC complexes and test weather TRIM5α blocks infection by binding to the HIV-1 capsid. This CA-NC complex was synthesized in vitro by transfecting BL-21 star E. Coli cells with CA-NC HIV-1 recombinant expression plasmid and inducing the cells to produce protein. The protein was then purified and concentrated. A binding assay for TRIM5α and capsid was conducted via sucrose gradient centrifugation followed by western blotting to verify binding. Binding between TRIM5α and capsid was evident. Infection was tested using dog cell lines with and without TRIM5α. These mammalian cells were infected with GFP recombinant HIV-1. As the HIV-1 concentration was increased, the level of infection increased for cells without TRIM5α. The level of infection did not increase from about 0% when cells with TRIM5α were infected with increasing concentrations of HIV-1. These results suggest that binding of TRIM5α to HIV-1 capsid restricts infection of HIV-1. Future experiments can test individual amino acid differences between human TRIM5α and monkey TRIM5α. A study of mutations in this protein can lead to therapeutic implications in the treatment of HIV-1.

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Characterization of AP2 Transcriptional Regulator Knockouts in the *Plasmodium yoelii* Rodent Malaria Model

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Every year, 300-500 million cases of malaria occur worldwide and lead to nearly a million deaths, mostly occurring in sub-Saharan Africa (WHO, 2010). Malaria is caused by apicomplexan parasites in the genus *Plasmodium*, which are transmitted through an arthropod vector, the female *Anopheles* mosquito. One of the major areas of research is to understand the malaria lifecycle, particularly the role of the Apicomplexan AP2 family of DNA-binding proteins (Painter et al., 2011) in gene regulation. The ApiAP2 family is highly conserved across all malaria parasites, making it a target for possible therapeutic approaches, especially since the AP2 family is most closely related to AP2 transcription factors of plants.

Because human malaria experimental models are experimentally difficult, we use the rodent malaria model *Plasmodium yoelii*, which is relatively easy to manipulate genetically. In this study, we attempted to delete seven AP2 genes and characterized candidate parasite knockout lines. We investigated AP2 function in vivo in two hosts of *P.yoelii*, the mouse, and the mosquito, *Anopheles stephensi*. Four knockout lines were confirmed, and of these, one AP2 gene knockout (KO) parasite line was significantly less virulent ($p<0.0001$) and resulted in survival of all mice infected. Studies in the mosquito host are ongoing. These studies confirm the importance of AP2 genes for the viability of *Plasmodium.* Further studies will determine whether the AP2 can serve as important targets for anti-malarial drug development or be useful for creating attenuated vaccine strains to induce protective immunity to malaria.

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Examination of Clostridium difficile Transmission in New York City Hospitals using Ribotype Analysis

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Clostridium difficile is the most common cause of antibiotic-associated diarrhea in hospitals. The increase in the incidence and severity of this spore-forming, anaerobic bacterium across the United States has made it a leading hospital-acquired infection. In the New York area, the increase in incidence and severity is related to the introduction of a hypervirulent, epidemic North American strain. To identify the epidemic strain, various typing methods have been employed. In this experiment, ribotype automated sequencer-based capillary electrophoresis (RACE) is used to classify different strains of *C. difficile*. It may also used to understand *C. difficile* infection control within and among 5 different hospitals in the New York area. In order to collect this data, 81 anonymous stool samples were collected from these hospitals. The stool samples were mixed with ethanol, plated on selective media, and cultured anaerobically. DNA was obtained from the cultures and was tested for markers of the epidemic strain (a toxin gene tcdC deletion as well as accessory binary toxins) using PCR to amplify these DNA fragments. For further information on each strain, RACE was used to create a ribotype bar-code. Combining all of this data, a dendrogram was created to map out the spread and relationship of the different strains of *C. difficile*. We found that the epidemic C. difficile strain is spreading both within and between hospitals, showing that the epidemic strain is endemic in New York City.

Regulation of Fat1 Cadherin Expression

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Cardiovascular disease is the leading cause of death, disability, and healthcare expense in the western world. Vascular remodeling is implicated in the pathogenesis of atherosclerosis, restenosis following angioplasty and transplant arteriosclerosis. The molecular mechanisms behind vascular remodeling in disease remain incompletely understood. The Fat1 cadherin regulates vascular remodeling, inhibiting vascular smooth muscle cell (VSMC) growth, and promoting VSMC migration. Studies indicate that expression of the *fat1* gene is regulated at the transcriptional level. We seek to identify the *fat1* promoter region and the mechanisms behind its transcriptional regulation, especially its activation by serum.

A 20kb genomic fragment upstream of the *fat1* start codon was inserted into a pGL3 luciferase reporter construct. Restriction digests were performed to produce deletions of the 20kb *fat1* insert. Transfection of these plasmids into rat arterial smooth muscle cells (RASMC) was used to assess promoter activity. Using this strategy we identified a 4kb region that shows high promoter activity. Our results suggest that the promoter and transcription start site are in the proximity of a HindIII site located 1kb from the 3' end of this fragment.

Treatment with fetal bovine serum (FBS) induces *fat1* expression and does not modify *fat1* mRNA stability. Luciferase assays and western analysis of transfected RASMCs treated with inhibitors of kinases involved in growth factor signaling suggest that the Src and PI3K kinases promote *fat1* transcription, whereas MEK and P38 kinases are not involved.

Further studies are necessary to identify transcription factors implicated in *fat1* expression, and determine the relevance in vivo.

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Studies of the Endoplasmic Reticulum Chaperone Pdi1p under Stress in *Saccharomyces cerevisiae* Using Fluorescence-based Imaging Techniques

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The endoplasmic reticulum (ER) must necessarily cope with stress: conditions that promote the accumulation of misfolded proteins in the ER lumen. ER stress is managed via the unfolded protein response (UPR), a signal transduction pathway that results in increased levels of ER chaperone proteins. Chaperone proteins are capable of aiding newly synthesized incoming ER proteins with their folding process.

In this work, Pdi1p, an ER chaperone in *Saccharomyces cerevisiae*, was studied using fluorescence-based imaging. To this end, a plasmid vector containing an insert coding for the fluorescent protein sfGFP (superfolder GFP) was appended with a terminal HDEL sequence for ER retention. Pdi1p was chromosomally tagged with sfGFP through homologous recombination, an action confirmed by Western blotting. *PDI1* is essential for yeast viability, indicating the GFP fusion does not grossly impair Pdi1p function. However, yeast expressing Pdi1p-sfGFP-HDEL were more vulnerable to tunicamycin-induced stress than wild type yeast. Imaging revealed that under conditions of dithiothreitol-induced stress, yeast cells express elevated levels of Pdi1p, consistent with UPR induction. Furthermore, experiments using fluorescence recovery after photobleaching (FRAP) indicate that the mobility of Pdi1p dramatically decreases under conditions of misfolded secretory protein accumulation (induced by tunicamycin or dithiothreitol). This is the first study of Pdi1p function in living cells and reveals that Pdi1p binding of client proteins significantly increases during general stress. Our findings suggest Pdi1p plays a major role in sequestration of unfolded proteins and that Pdi1p, like mammalian BiP, may be a live cell reporter of the global unfolded protein burden.

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Relationship of β-Tubulin Isotypes and Drug Resistance in Triple Negative Breast Cancer Cells

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Triple negative breast cancer cells are estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) negative and therefore lack the targets for many therapeutic agents. They can be extremely aggressive and difficult to treat. It has been shown that βIII-tubulin, one of the six isoforms of β-tubulin, is strongly associated with drug resistance and aggressive tumor growth (McCarroll et. al 2010). We have investigated whether the relative amounts of the different isoforms of β-tubulin play a role in drug resistance to three different drugs; Taxol, epothilone B, and ixabepilone. In addition, we analyzed the levels of EGFR (epidermal growth factor receptor) and EpCAM (epithelial cell adhesion molecule), ER, and HER2 in the same cell lines. Our analysis used western blotting techniques with antibodies for βI, βIII, βIV, and βV-tubulin, as well as for EGFR and EpCAM. Six different cell lines were studied; MCF-7, HS578T, MDA157, MDA468, BT549, and HCC1500. Cytotoxicity assays were used to measure the IC_{50} values of our four triple negative cell lines. A correlation existed between the increased amount of βIII and βIV-tubulin and increased resistance as seen in Hs578T as compared to MDA468, the latter having less βIII and βIV and being less resistant to all three drugs. However, no strong trends could be seen across all cell lines in terms of drug resistance, β-tubulin isoforms, and EGFR and EpCAM levels. Other factors, unknown to us at this time, may play a role in modifying drug resistance.

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Title: Acute Effects of 17beta-Estradiol on Dopamine Transmission in the Nucleus Accumbens

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Introduction: Sex differences in dopamine transmission are believed to mediate the greater susceptibility to addiction commonly observed in females as compared to males in response to cocaine or other psychostimulant drugs. However, the mechanisms underlying these differences are unknown. Several laboratories have examined how chronic 17β-estradiol (E2) administration regulates neurotransmission in the dorsal striatum, but few have investigated the rapid effects of estrogens in the nucleus accumbens (NAcc), a structure implicated in drug addiction. Here we propose to measure acute effects of E2 on dopamine transmission in the NAcc.

Methods: Using fast-scan cyclic voltammetry, real-time changes in dopamine concentration were recorded in the NAcc core or shell of anesthetized rats in response to stimulation of the ventral tegmental area at variable frequencies and amplitudes. On the day of experiment, baseline DA responses to stimulation were recorded; animals were then injected with S-raclopride in combination with vehicle or E2. Effect of vehicle or E2 on raclopride potentiation of DA transmission was evaluated 30 minutes post-injection.

Results: We observed no difference in baseline dopamine transmission between ovx females and males, suggesting that the potentiated dopamine transmission observed in intact females is due to the presence of ovarian hormones. S-raclopride-induced potentiation of DA release was significantly higher in E2-treated ovariectomized females than in vehicle-treated animals. No differences were observed between NAcc core and shell.

Conclusions: These results, when combined with future studies on long-term E2 effects in NAcc, may help to explain the greater susceptibility to drug addiction that is observed in females.

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Loss of *ALL2*, a homologue of *ALL1*, alters the virulence, life span and is upregulated under low-glucose environment

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Cryptococcus neoformans is an important pathogen in immunocompromised patients such as those with AIDS. Phenotypic switching of *C. neoformans* from smooth (SM) to mucoid (MC) variant resulted in down regulation of both *ALL1* and *ALL2*. Previously seen, the loss of *ALL1* confers a hypervirulent phenotype and produced a larger polysaccharide capsule than wild type SM. *ALL2* shares >85% homology with *ALL1* in all but the carboxy terminal. The purpose of these experiments was to investigate the phenotype of *all2*Δ mutant. Null mutant of *ALL2* also shared the hypervirulent and capsular phenotype of *all1*Δ. Replicative life span (RLS) analysis was done by dissecting the buds of virgin cell using a micromanipulator and showed that *all2*Δ and *all1*Δ*all2*Δ have increased life spans as compared to wild type [Median life span of 72.5 (*all2*Δ) and 79.5 (*all1*Δ*all2*Δ) vs 62 (SM), $p < 0.0001$]. To confirm the role of *ALL2* in alternating virulence and life span, the gene function was restored by complementing *all2*Δ with *ALL2*. The compliment strain, Δ*all2*+p*act1-ALL2* were produced by the amplification and ligation of *ALL2* and actin promoter (p*act1*) from RC2-SM and cloned into pJAF13 (NAT plasmid). The cloned plasmid was linearlized and randomly inserted into *all2*Δ cells by biolistic transformation. Eighteen of the 20 transformants were positive for *Pact1-ALL2* as determined by PCR and relative gene expression of *ALL2* by real time PCR in Δ*all2*+p*act1-ALL2*. Later, we investigated the expression of *ALL2* and *ALL1* in low glucose conditions (0% and 0.2%) and found that both genes are upregulated under low glucose environment. Our data identified a set of genes that alters the virulence, capsule and life span of *C. neoformans*.

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Effects of TLR agonists and Hormones on the Permeability of Polarized Epithelium to HIV

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Introduction: Genital susceptibility to HIV varies due to host factors, including infections and pregnancy. The goal of this project was to examine the effects of toll-like-receptors (TLR) agonists and hormones on the genital epithelial barrier. **Methods:** HEC-1-A cells (endometrial cell line) were cultured in Transwell inserts under polarizing conditions and exposed to TLR agonists 1-9 for 24 h after good polarization was observed. Following removal of the agonist by washing, HIV-1_{BaL} (40 ng p24) and Jurkat-Tat-CCR5 cells (a T cell line) were added to the apical and basolateral compartments, respectively. Transepithelial resistance (TER) was monitored and basolateral supernatants were collected daily for quantization of HIV-1 p24 antigen by ELISA. The effects of medroxyprogesterone acetate and 17β-estradiol on permeability were assessed using the same methods as described above. The effect of hormones on HEC-1-A cell growth was also examined by exposing cells to hormones during plating. **Results:** Exposure to TLR agonists resulted in increased TER in response to TLR-3HMW, no significant difference in p24 levels and a NFκB response to 3HMW, 3LMW, 7, and 9 agonists. The hormone medroxyprogesterone acetate decreased cell growth when present during plating and increased permeability when added after full confluence was reached. 17β-estradiol had no effect on cell barrier integrity. **Discussion:** These findings indicate the effects of external/internal factors on the vaginal epithelial. This can be related to susceptibility to HIV expected due to progesterone drug treatment in the form of birth control. Permeability change during certain infections can also cause an increase in HIV susceptibility.

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An animal model of effort based decision-making

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The nucleus accumbens (NAc) is a group of neurons located in the forebrain. Evidence suggest that NAc neurons encode both the action leading to rewards and the rewards themselves but the exact role of the NAc remains undiscovered. It has been proven previously that dopamine plays a role in the decision to exert effort to seek reward. Because of this, it is possible that the NAc contributes to the decision of which reward to pursue, especially when effort is required to obtain reward. In this experiment, we test the hypothesis that NAc neurons encode the value of the target when animals choose among targets associated with different reward size or effort cost. Rats perform a decision making task that requires the selection of approach targets (levers to press). The trials began with the presentation of an auditory cue that coincides with the extension of one lever or two levers depending on the trial. Lights were also illuminated above each lever. The rats were first put through high effort (8-16 lever presses) and low effort (one lever press) options with the same reward. After the rats became accustomed to that system, they had to choose between a low-reward, high reward option with the same number of lever presses (one lever press). The rats' choices showed that they were able to make their decisions based on reward and effort. In future experiments, single neurons in the NAc core will be recorded while the rats perform this decision-making task.

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Par 1 expression is altered in experimental models of kidney disease

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Focal Segmental Glomerulosclerosis (FSGS) and Diabetic Nephropathy (DN) are the leading causes of acquired end-stage kidney disease (ESKD) in children and adults, respectively. Both FSGS and DN are diseases that affect the kidney's filtering unit (glomeruli) causing scarring, and are characterized by loss of protein in the urine called proteinuria. Key features in FSGS and DN include damage to the glomerular epithelial cell, the podocyte, with effacement of podocyte foot processes. The podocyte is a highly structured cell with an apical cell body that extends foot processes with apical, basal and junctional domains. Par 1 is a serine/threonine kinase that plays a role in establishing cell polarity in columnar epithelial cells and neurons and is expressed in the developing kidney and in podocytes. Suppression of Par1 signaling in podocytes in vitro led to changes in cell shape, suggesting Par1 may play a role in maintaining podocyte structure.

Our hypothesis was that podocyte polarity expression would be altered in the setting of proteinuric kidney disease. Expression of Par1a/b was examined in rodent models of FSGS and diabetic nephropathy (DN). Immunofluorescence was performed on cryosections of diseased 20 week adult mice that were injected with streptozotocin (STZ) to induce Diabetic nephropathy or adriamycin (ADR) to induce glomerulosclerosis. Adult rats were injected with puromycin aminonucleoside (PAN) to induce nephrosis. Controls (both mice and rat) were injected with vehicle. ADR and PAN nephrosis induce FSGS-like disease. Results showed an altered expression of Par1a in all forms of proteinuric kidney disease whereas Par1b expression appeared decreased in diabetic and adriamycin nephropathy but was focally increased in PAN specimens. This suggests that polarity may be altered in these diseases. Future experiments include examining expression levels with quantitative methods such as Realtime PCR and Western Immunoblotting. In addition, we are in the process of generating a transgenic mouse model to turn off Par1 in podocyte as well as examining Par1 expression in human kidney samples.

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Understanding the Role of Intronic Cis-acting Elements in the Splicing of MacroH2A1 Variants

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The histone variant macroH2A replaces the canonical histone H2A in nucleosomes in specific regions of the genome in order to regulate gene expression. Two splice variants of macroH2A1, macroH2A1.1 and macroH2A1.2, are encoded by mutually exclusive splicing of two alternative exons. Most normal human cells express similar levels of macroH2A1.1 and macroH2A1.2. However, work from our lab has shown that alternative splicing of macroH2A1 pre-mRNA, leading to a decrease in macroH2A1.1 expression, occurs in a variety of cancers. Additionally, ectopic expression of macroH2A1.1 represses cancer cell growth and induces senescence in a splice variant-specific manner. Therefore, it is important to determine the mechanism that regulates macroH2A1 splicing and determine how this mechanism is modified in cancer cells. In order to identify the cis-acting sequences that regulate macroH2A1 splicing, we designed a macroH2A1 minigene which includes three introns of 600 base pairs each flanking the alternative exons. In A549 lung fibroblast cells which only express macroH2A1.2, the macroH2A1 minigene only expresses the macroH2A1.2 spliced transcript. However, in MG-63 osteosarcoma cells which normally express both macroH2A1.1 and macroH2A1.2, the minigene still only expresses macroH2A1.2. This suggests that the macroH2A1 minigene is missing critical cis-acting sequences that are necessary to accurately splice macroH2A1.1. Interestingly, several highly conserved elements exist in the introns flanking the alternative exons of this gene. By applying our mingene splicing assay we are systematically analyzing the contribution of these ultra-conserved regions to the regulation of macroH2A1 splicing.

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Aberrant regulation of GluR2 mRNA abundance in dendrites of Fragile X neurons

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Fragile X Syndrome (FXS) is the most common form of inherited mental retardation, occurring in ~1/3600 births (Crawford et al., 2002). FXS occurs when the gene coding for Fragile X Mental Retardation Protein (FMRP) is silenced. FMRP is a RNA binding protein, which regulates mRNA transport, stability and translation in dendrites (Corbin et al., 1997; Ohashi, 2002). Loss of FMRP leads to exaggerated Group 1 metabotropic glutamate receptor (mGluR) long-term depression (LTD). Stimulation of Group 1 mGluRs in hippocampal neurons have been shown to recruit mRNA encoding the AMPAR subunit, GluR2 to dendrites (Grooms et al., 2006). Because *Fmr1* KO mice exhibit exaggerated group 1 mGluR LTD and Group 1 mGluR activation is known to increase GluR2 mRNA targeting to dendrites (Grooms et al., 2006), we examined whether GluR2 mRNA abundance in dendrites will be increased in neurons from *Fmr1* KO mice. Preliminary studies have demonstrated an increase in GluR2 mRNA in hippocampal dendrites of *Fmr1* KO mice. Because FMRP is known to not bind GluR2 mRNA directly, we next examined whether the only RNA binding protein known to bind GluR2 mRNA, CPEB3 (Huang et al., 2006), is also dysregulated in dendrites of hippocampal neurons from *Fmr1*KO mice. We found that like GluR2 mRNA abundance, CPEB3 protein abundance is elevated in dendrites of hippocampal neurons. Using fluorescent immunocytochemistry, we discovered that acute knockdown of CPEB3 in hippocampal neurons reduces GluR2 mRNA abundance in dendrites therefore suggesting that CPEB3 is responsible for GluR2 mRNA transport to dendrites. We next seek to address whether the changes observed in CPEB3 and GluR2 mRNA abundance in dendrites of *Fmr1* KO hippocampal neurons are casually related.

Does the Overestimation of the Roche HCV viral load persist at the lower limits of detection as compared to the Abbott Assay? A clinically important question.

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Telaprevir and Boceprevir are two recently approved drugs for the treatment of Hepatitis C virus (HCV). These require a patient's viral load (the amount of HCV RNA) to be monitored by a PCR assay with a lower limit of quantification (LLOQ) of 25 IU/mL and a limit of detection (LOD) of 10 IU/mL. Roche COBAS AmpliPrep/COBAS Taqman and Abbott RealTime HCV are two FDA approved HCV real-time polymerase chain reaction (RT-PCR) viral load assays utilized in the US health care system. The drugs pose a problem as the Roche assay's LLOQ is 43 IU/mL. The Virology laboratory at Montefiore Medical Center uses the Roche assay to determine the HCV viral load. We asked if the Roche assay is sensitive enough to monitor patients being treated with the drugs. A previous comparison study done in our laboratory demonstrated that the Roche assay is consistently higher than the Abbott assay across a dynamic range of 0-4 log IU/mL. The object of this study is to test if this overestimation would be observed at the lower viral load range. Clinical samples were collected and run in both assays to establish a baseline on both instruments. The same dilution factor was then applied to reach the LLOQ. Preliminary data suggest that the Roche assay results in higher viral loads at the lower range. A conversion factor has yet to be determined. Our results are not in agreement with the published literature[i] suggesting that the overestimation of the Roche assay occurs exclusively in undiluted clinical samples. The literature suggests this is due to a biochemical interaction with a blood component that can be diluted out. However our preliminary data show the overestimation occurs even after dilutions to lower viral load levels. The goal of our work is to clarify the lower range in an attempt to utilize the Roche assay for patients on these drugs. This information is important for physicians treating patients with chronic HCV on these newer medications.

[i] Chevaliez et al., Overestimation and Underestimation of Hepatitis C Virus RNA Levels in a Widely Used Real-Time Polymerase Chain Reaction- Based Method. *HEPATOLOGY* 2007; 46:22-31.

Abstract:

Engineering Soluble T Cell Receptors as Therapeutic Molecules

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HIV infected cells present viral peptides in the context of MHC proteins expressed on their surface which are recognized by T Cell Receptors (TCR) expressed by cytotoxic T cells which then kill them. Our lab seeks to understand the structural basis by which TCRs recognize MHC + cognate viral peptide and also harness the TCR – MHC+peptide interactions to develop novel therapies to kill HIV-infected cells.. The advantages to using a soluble TCR to recognize and eliminate HIV cells are that they are highly specific, highly sensitive, detect infection early in the replication cycle and will allow for the specific targeting and elimination of infected cells before they propagate viral progeny. By conjugating the TCRs to a toxin, we can delver these toxins specifically to HIV-infected cells and eliminate HIV infected cells. Using an engineered TCR, we have been able to produce soluble TCRs in mammalian cells and subsequently isolate purified protein from the cell supernatant through affinity purification. In an attempt to increase soluble TCR production, we have cloned the TCR α and β genes on a single plasmid with a “self cleaving” 2A peptide to allow for equimolar expression of the two genes enabling efficient production of the TCR heterodimer. . To further increase protein production, we have incorporated this construct into a lentiviral expression vector to generate stably transfected cells producing high concentrations of the TCR.

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Purification of *A. fumigatus* Dipeptidyl Peptidase

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The prevalence of asthma is increasing worldwide and can be triggered by a variety of factors. Inhaled fungal spores are a trigger for asthma in some patients. Proteases recently have been recognized as playing a role in inducing the asthma response. This lab had previously created monoclonal antibodies (MAbs)154 and 293, which bind to DPP, a protease in *Aspergillus fumigatus,* and MAb 154 prevents inflammation in a mouse model of fungal asthma. Based on these experiments, we want to examine if DPP induces sensitization to *A. fumigatus* spores. If DPP sensitizes mice to *A. fumigatus*, then the antibody has potential to be an adjunctive therapy for asthma in patients whose exacerbations are triggered by exposure to *A. fumigatus*. mRNA was expressed with a His tag in *Pichia pastoris* to make recombinant (r)DPP. Time course analysis showed the best protein production in culture at 72 hours. DPP-His was purified using a nickel sepharose column and an average concentration of 0.5 mg/ml was obtained in the peak fractions. Purified rDPP will now be used to sensitize mice, who will then be challenged with *A. fumigatus*. Mice will be examined for differences in the inflammatory response to determine the effect of DPP.

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Variable Expression of Argininosuccinate Synthetase Protein and Correlation with Novel Therapeutic ADI-PEG20 in Osteosarcoma

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The amino acid arginine is involved in protein synthesis and tumor metabolism and is essential for the growth of human cancer cells. Pegylated arginine deaminase (ADI-PEG20) is a novel therapy that lowers extracellular arginine levels and has shown evidence of clinical efficacy and low toxicity in patients with tumors lacking argininosuccinate synthetase (ASS1) protein expression. Previous studies have demonstrated the effectiveness of ADI-PEG20 in cancer cell lines with diminished or absent ASS1 protein including melanoma and hepatocellular carcinoma. Breast cancer and lung cancer cell lines, both of which frequently maintain strongly positive ASS1 protein expression, continued to proliferate in the presence of ADI-PEG20. ADI-PEG20 sensitivity has not been previously evaluated in osteosarcoma.

Because ADI-PEG20 appears to correlate with ASS1 protein expression, a cohort of osteosarcoma tissue sections (n=171) was stained immunohistochemically to determine the presence or absence of ASS1 protein. Sixty-four percent (n=109) of the sections stained indicated negligible or no staining for ASS1 protein suggesting that osteosarcoma might be a candidate for ADI-PEG20 sensitivity. Western blots were performed on patient-derived osteosarcoma cell lines (n=18) to determine levels of ASS1 protein expression prior to treatment with ADI-PEG20. Of the 18 cell lines studied, four cell lines had noticeably diminished ASS1 protein expression.

Cytotoxicity assays are being performed on the four cell lines with decreased ASS1 protein expression in conjunction with additional osteosarcoma cell lines exhibiting high ASS1 protein expression. If the cytotoxicity assay results correlate with ASS1 protein expression, ADI-PEG20 treatment of osteosarcoma xenograft mice will be pursued.

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Determining the role of Mck1 and Kns1 in the Target of Rapamycin pathway

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The Target of Rapamycin (TOR) signaling pathway is responsible for regulating signals involved in gene expression, metabolism, and cell growth. Even to date, many of the downstream components of the TOR kinase cascade that regulate growth have not been identified. A screen for regulators of RNA polymerase III transcription uncovered two protein kinases, *MCK1* and *KNS1*, that function downstream of the TORC1 complex and are required for transcriptional repression of both ribosome and tRNA biosynthesis. To determine the role of the *MCK1* and *KNS1* kinases in TOR signaling, synthetic genetic methodology was used to delete both kinases in an ordered array of ~5,000 viable gene deletion mutants (representing ~80% of all *S. cerevisiae* yeast genes). Sensitivity to rapamycin, an inhibitor of TOR kinase, was used to identify triple gene deletions that affected the TOR pathway.

A mini-array comprised of ~300 rapamycin-sensitive gene deletions was generated with the single *MCK1* and *KNS1* gene deletions in addition to the double kinase deletion strain. The mini-array was used to examine the effect of each individual kinase deletion on TOR sensitive phenotypes such as survival after starvation, metabolism, autophagy, and rapamycin sensitivity.

Although the cellular role of *KNS1* is currently poorly defined, these studies have uncovered a link between *KNS1*, the MAPK cell integrity pathway and TOR signaling, and mitochondrial function.

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The Role of DNA Methyltransferase 3b (Dnmt3b) In Heart Development

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During embryogenesis, the heart is the first organ to develop. Heart development is a complex process that is regulated by multiple levels of regulation, including genetic, environmental and epigenetic processes (Srivastava and Olson, 200). Epigenetics modifications regulate gene expression by changing local DNA structure without altering the nucleotide sequence (Godfrey et al. 2007). These modifications include modification of histones, chromatin packaging, and DNA methylation (Gluckman et al. 2009). DNA methylation is the addition of a methyl group to the carbon 5 of cytosine. This process is mediated by a group of enzymes called DNA methyltransferases which are essential for normal embryonic development (Geiman and Muegge, 2009).

During early embryogenesis, with the exception of imprinted genes, the mammalian genome is stripped of its epigenetic modifications. The re-establishment of the epigenome during embryonic development is mediated by de novo DNA methyltransferases, including DNA methyltrasferase 3a (Dnmt3a) and DNA methyltrasferase 3b (Dnmt3b).

Animal studies have shown that loss of Dnmt3b results in dysfunctional development and embryonic death, with 70% of embryos dying between embryonic day (E) 13.5 and E16.5 and 30% of embryos dying by E11.5 (Okano et al., 1999). However, the role of Dnmt3b in heart development has not been studied.

We hypothesize that Dnmt3b plays an essential role in heart development. Identifying the role of Dnmt3b in heart development will not only provide us with a better understanding of heart development but will also provide us with a better understanding of the congenital heart defects that result in disruption of the development process.

To test this hypothesis a transgenic mouse model was created in which Dnmt3b was selectively knocked out in specific cell populations within the heart. Gross morphology was studied immediately following dissection and then histological serial sectioning, followed by haematoxylin and eosin staining, was performed to study the morphology of the inner structures of the heart.

When Dnmt3b was knocked out of the endocardium embryos exhibited hemorrhaging and the heart appeared abnormal. Preliminary histological serial sectioning of Dnmt3b-/- embryos at E13.5 indicates a potential role for Dnmt3b in pulmonary valve development. Additional endocardial specific specific Dnmt3b-/- embryos will be investigated as well as additional cardiac specific Dnmt3b-/- embryos to further investigate the role of Dnmt3b in heart development. Finally, we will identify targets of Dnmt3b in heart development to identify genes and pathways affected by DNA methylation in heart development.

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Identification of Novel Proteins of the Infectious Apparatus of *Encephalitozoon hellem*

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Microsporidia infect host cells using a unique mechanism in which they discharge a polar tube, which pierces the host cell, and allows for the microsporidia to transfer its cytoplasm into the host. The polar tube is made up of multiple proteins that are thought to work together in the infectious process (3). Further highlighting the composition of the polar tube and any novel proteins would allow for a more comprehensive understanding of the microsporidian infectious process. After mass spectrometry, over thirty candidate hypothetical proteins of polar tube extracts were identified. This study describes the expression of recombinant proteins identified by this proteomics approach.

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Baicalein Abrogates Inflammation Through cdx2/PXR Pathway in Murine Colitis

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Baicalein, a flavanoid originating from the root of *scutelleria baicalensis,* and baicalin (*O*-glucuronide of baicalein), are polyphenols with potent anti-inflammatory and anti-cancer properties. There is hepatic conversion of baicalein to baicalin. Baicalin is modified to baicalein through microbial β-glucuronidase. Pregnane X Receptor (PXR), a master regulator of drug metabolism and inflammation, is abundantly expressed in the gastrointestinal tract. Here, we investigated whether these flavonoids inhibit inflammation through a PXR-specific pathway in the gut. Regulation of PXR and consequences of its activation by these flavonoids were evaluated in human colon carcinoma cells and in vivo, using *wild-type*, *pxr-null*, and *humanized (h) PXR* C57Bl/6 mice. Baicalein, in contrast to baicalin, induces PXR through cdx2 in human colon carcinoma LS174T cells in vitro and murine colon in vivo. Baicalein, in contrast to baicalin, activates PXR. Both flavonols abrogate DSS-mediated colon inflammation in vivo; however, only Baicalein abrogates inflammation in PXR-functional but not in PXR-deficient mice. Inh1, a novel microbial specific β-glucuronidase inhibitor that prevents baicalin from converting to baicalein, abolishes the effect of baicalin on inflammation. Finally, functional cdx2 binding sites mapped to specific proximal sites on the PXR promoter.

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HSP90 Inhibition in Mouse Hippocampus CA1 Region with Resulting Effects on Long Term Synaptic Depression

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Introduction: Long term synaptic depression (LTD) occurs both through changes in neuronal ion gate channel permeability, as well as, through locally synthesized protein production in dendrites. These locally synthesized proteins can be responsible for non-transient depression in neurons. It has been shown that when translation of these local proteins are inhibited, a reduction in the magnitude of LTD occurs. We sought to determine if a similar reduction in LTD magnitude occurred when protein folding was inhibited in these cells. The HSP90 inhibitor radicicol was used to test this hypothesis.
Methods: Electrophysiological field recordings were taken in the CA1 region of mouse hippocampal slices. Depression was induced chemically with the drug DHPG. The HSP90 inhibitor was the drug radicicol.
Results and Discussion: Inhibition of HSP90 protein significantly reduces DHPG-induced long term depression in the CA1 region of mouse hippocampus, without affecting basal synaptic transmission. DHPG chemically induces LTD through activating MGluR1 and MGluR5 receptors in neurons. Our results suggest that blocking the HSP90 chaperone inhibits DHPG's ability to induce long term depression through interfering with the endocytosis of AMPA receptors on the post-synaptic terminal. We believe radicicol's effect is postsynaptic, given that MGluR1 receptors are specific to postsynaptic terminals and, in Ca1 MGluR1 dominates over MGluR5. The exact mechanism of this reduction in LTD is unknown and requires further investigation on the single cell level with experiments which selectively block MGluR1 and MGluR5 receptors as well as tests to see whether the same reduction in LTD is seen in other regions of the hippocampus.
Conclusion: Understanding the mechanisms of Long Term Synaptic Depression holds value in understanding human memory and learning. Importantly, discovering methods to reduce or extend LTD are of significant interest. Through applying the HSP90 inhibitor, radicicol, to a DHPG induced LTD, a great reduction in long term depression was observed. Investigating the precise mechanisms behind this reduction in depression suggests a postsynaptic mechanism, but does not exclude presynaptic influence.
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Discovery of Novel Proteins that Mediate Subcellular Trafficking of Organic Anion Transport Protein 1a1 (oatp1a1)

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Hepatocytes play a vital role in detoxification and removal of xenobiotic compounds, including organic anions, from the circulation. This process is mediated by specific cell surface transporters, one of which is organic anion transport protein 1a1 (oatp1a1), a protein that has been discovered by our laboratory. Prior studies have shown that oatp1a1 can be phosphorylated at two serine residues on its cytoplasmic tail, and this results in its internalization and loss of transport activity. Our current efforts are aimed at discovering proteins that mediate this internalization process, and subsequent loss of cellular transport activity. cDNA encoding the oatp1a1 cytoplasmic tail (aa 624-670) was inserted into the pGEX6p-1 GST fusion protein expression plasmid. Site-directed mutagenesis of the nucleotides encoding the serine residues at positions 634 and 635 was performed to produce phosphomimetic and nonphosphorylatable derivatives in which these serine residues were replaced by glutamic acid or alanine, respectively. Plasmids were expressed in *E. coli* and synthesis of GST-oatp1a1 hybrid proteins was confirmed by Western blot using antibodies specific for GST and oatp1a1. These proteins were purified by binding to GSH-agarose beads. In future studies, these agarose bound proteins will be incubated with rat liver cytosol to identify interacting proteins that may be required for internalization of phosphorylated oatp1a1.

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Characterization of *Mycobacterium smegmatis* mutants resistant to the first line anti-tuberculosis drug Isoniazid

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Tuberculosis has always been a known problem all over the world but the issue has increased now that it is showing resistance to drugs that are seen as very effective in killing it. The first line drug isoniazid (INH) was introduced in 1952 and its introduction was so effective that it caused the closing of the tuberculosis sanatoriums that until that point were the only form of treatment of tuberculosis. The use of anti-tuberculosis drugs led to the emergence of drug-resistant strains of *Mycobacterium tuberculosis,* the causative agent of tuberculosis. As more and more drug-resistant strains of *M. tuberculosis* have been isolated, the investigation of how *M. tuberculosis* gains drug resistance began. I am studying INH resistance in *Mycobacterium smegmatis* because its doubling time is $1/7^{th}$ of *M. tuberculosis* and it also displays similar patterns of drug resistance. I have been able to isolate INH-resistant mutants in 3 different media at a frequency of $2x10^{-5}$ for Tryptic Soy Broth, $2x10^{-5}$ for Mueller Hinton and $8.8x10^{-7}$ for Middlebrook 7H9. Previously, INH-resistant mutants that also display temperature sensitivity and serine/glycine auxotrophy have been isolated. These mutants had mutations in *ndh*, a gene encoding NADH dehydrogenase type II. The correlation between *ndh* mutation and serine/glycine auxotrophy is puzzling, so my project has been to repeat the isolation of these mutants and analyze them with the hope to further understand how mycobacteria gains drug resistance.

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Combating Fungal Infections with Nanoparticle Encapsulated Amphotericin B

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Amphotericin B (AmB) is a commonly used antifungal drug which has severe side effects that can lead to serious complications. In the past, the Nosanchuk lab has successfully used silane-based nanoparticles as a therapeutic delivery mechanism. The nanoparticle's size theoretically allows it to slip through the fungi's extracellular biofilm and directly target the microbe, while concomitantly limiting drug toxicity.

Nanoparticle encapsulated AmB (np-AmB) preparations were prepared according to developed protocols and the release kinetics of the drug were studied. Different concentrations of both solubilized AmB (s-AmB) and np-AmB were delivered to standard populations of *Candida albicans (CA)*. Metabolic assays with XTT salt were taken at specified intervals. In a parallel experiment, each concentration of the AmB was applied to an established *CA* biofilm, and the yeast cells were then collected and plated on YPD agar to compare growth rates. The results describe a steady release of AmB from the nanoparticles and show the np-AmB to be as efficient as the solubilized AmB in the inhibition of *CA* growth. Finally, the np-AmB was delivered to twelve strains of *Candida*, and the np-AmB exhibited similar or increased efficacy compared to the s-AmB.

The study suggests a novel method of delivery of AmB. For treatment of focal fungal disease, such as in an infected intravenous catheter or a cutaneous infection, np-AmB, which slowly and steadily releases the drug, would likely have less toxicity than s-AmB. In addition, the study demonstrates encapsulation of AmB by our methods does not inhibit the efficiency of the drug. Hence, the np-AmB is a promising new formulation of a potent antifungal that can be adapted for diverse clinical purposes.

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Protein-Protein Association Between HERG Intracellular Domains and KCNE2

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Abstract:

Ion channel subunits encoded by human Ether-á-go-go Related Gene (HERG) and KCNE1 & 2 genes are known to interact and produce the rapid activating K^+ current (IKr) that is essential for normal myocardial repolarization during phase 3 of the cardiac action potential. The specifics of this interaction, however, are drawn from homologous models, since there is no definitive evidence as to the structure, interaction sites, and resultant conformational changes in the protein complex. Mutations in HERG and KCNE2 cytoplasmic domains may result in forms of Long QT Syndrome (type 2 and 6), characterized by a prolonged QT-interval measured by electrocardiogram, which predisposes affected individuals to experiencing life-threatening arrhythmias, syncope, and sudden death via ventricular fibrillation.[1] LQTS can be either congenital or acquired, and is estimated to affect ~45,000 Americans, resulting in the death of 4,000-5,000 citizens (mostly children and young adults) annually.[2]

In the present study, we investigated the interaction between HERG channel intracellular domains (HERG-ID) and KCNE2 in an attempt to identify cytoplasmic interaction sites to apply to structural and mutagenesis/electrophysiology studies later. Our primary results show positive association of HERG-ID (214-320 amino acid) with KCNE2 and was achieved through co-immunoprecipitation. This N-terminal region comprises PKA-dependent phosphorylation site (S283) and LQT mutation sites, suggesting its importance for the regulation of the channel behavior. This “pull-down” experiment is the first of many results needed to elucidate the mechanism of action for this channel that will later serve to develop reliable diagnoses methods and more advanced treatment of LQTS.

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The Role of Exo1 in DNA Damage Response and Proliferation

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HNPCC (hereditary nonpolyposis colorectal cancer), a common cancer predisposition syndrome that affects 1 in 200 individuals in the western world, may lead to the early onset of colorectal cancer. More than 90% of these cancers are associated with mutations in DNA mismatch repair (MMR) genes. Exo1, a non-essential gene, is the only known exonuclease involved in the MMR pathway, is involved in double strand break repair and is also involved in a variety of other pathways including tumor suppression and meiosis. Cell survival of mouse embryonic fibroblasts (MEFs) with or without the exonuclease function (EK), as well as MEFs without the Exo1 protein (null), are being assessed by performing clonogenic assays to determine if there is a phenotype associated with the mutant MEFs. Cell lines are viable when Exo1 is knocked out, but when Exo1 is knocked out along with Hus1 or Rad18--two other genes involved in DNA replication or recombination--the cells phenotype is sick/lethal. Therefore, double knockdown mutants will be generated by transfecting MEFs with shRNA lentiviral plasmids after determining which shRNA plasmids are most effective at knocking down the target genes. If WT and mutant MEF cell lines are stably transfected, clonogenic assays and other biochemical procedures to assess genomic stability will be performed to understand more about the pathways Exo1 is involved in and the associated phenotypes of the single and double mutants. Understanding these pathways in more depth will enable novel anti-cancer treatments to be studied and developed.

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Breast cancer is the second leading cause of cancer related deaths in women. This high mortality rate associated with breast cancer stems from the development of metastatic disease.
The metastasis cascade consists of a multi-step process that involves the spread of cancer cells from the primary tumor site to secondary organs and tissues through processes of invasion, intravasation, extravasation and survival/growth of cancer cells at new sites. Previous studies have shown that breast cancer malignancy can be enhanced through a paracrine loop interaction between breast cancer cells and tumor associated macrophages utilizing epidermal growth factor (EGF) and colony stimulating factor 1 (CSF-1). It was found that the EGF/CSF-1 paracrine loop between tumor cells and macrophages can be modified by different ligands secreted by breast cancer tumor cells. CXCL12 is a chemokine that has been found to stimulate invasion and it has been shown that CXCL12 stimulates tumor cell invasion *in vitro*, as well as playing a key role in the metastatic behavior of these cells *in vivo*. Recent work in our lab has determined that increased expression of CXCL12 by MTLn3 rat mammary adenocarcinoma cells can recruit additional macrophages and increase tumor cell invasiveness. Our hypothesis is the over-expression of chemokines by tumor cells can enhance invasiveness through stimulation of macrophages using the paracrine loop. The study will test this hypothesis by expressing different chemokines (CXCL12, CCL2, CCL4, CX3CL1, VEGFA and IL8), whose expression is correlated with poor prognosis, in MDA-MB 231 ATCC human breast cancer cells and evaluating whether or not there is an effect on invasiveness and metastasis. We have generated stable transductants of MDA-MB-231 cells carrying the CXCL12 expression constructs using two expression vectors, pLEX and pQCXIP. Our results from the ELISA validated the overexpression of CXCL12 in these transductants. Future work will be done in order to determine if expression of CCL2, CCL4, VEGFA, CX3CL1 and IL8 in the 231 ATCC and MTLn3 cell lines also increases tumor associated macrophage density and *in vivo* invasiveness.

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Sequencing and Evolutionary Dynamics of Asian and African Human Papillomavirus Genomes types 18 and 45

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Papillomaviruses (PVs) are a family of highly related viruses with circular double stranded DNA genomes. To date, 150 distinct HPV types have been assigned. A subset of HPV types have oncogenic potential predominantly causing cervical cancer. Human papillomavirus types 18 (HPV18) and 45 (HPV45) account for approximately 20% of all cervix cancer, and have a distinct biology from HPV16 - lesions caused by these types are often missed by Pap tests. Over 120 HPV18 and HPV45 samples isolated from patients in Asia (Taiwan, Thailand) and Africa (Rwanda, Burkina Faso and Zambia) have been analyzed by sequencing the URR/E6 regions; their variant lineages have been classified based on the single nucleotide polymorphism (SNP) patterns compared with the prototype sequences. In this study, we selected 15 HPV18 samples and 15 HPV45 samples for complete genome sequencing that represented different variant lineage or contained unique nucleotide variations. Samples were PCR amplified in 2 overlapping fragments, purified, submitted for sequencing, and assembled into complete genomes.

The complete genome for 1 HPV18 sample and 3 HPV45 samples were successfully sequenced, and 2 HPV18 samples and 2 HPV45 samples were partially sequenced. Following sequence alignment, 8 and 13 novel SNPs were identified from HPV18 and HPV45 samples, respectively, with changes distributed throughout the ORFs E1, E2, E5, E6, E7, L1, L2, and the URR. Phylogenetic analyses clustered sequenced samples into previously identified HPV18 and HPV45 variant lineages.

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Rbpms2 binds an essential regulator of germ plasm assembly and localizes to the PGCs

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Abstract
A key event in vertebrate embryonic development is axis specification to form the basic body plan. The first axis, the animal vegetal (AV) axis, is specified during oogenesis. In zebrafish, the first marker of the AV axis is a vegetally localized aggregate of organelles including ER, golgi and mitochondria called the Balbiani Body. Germ plasm is sequestered to the Balbiani body in organisms that specify the germline via inheritance of maternal RNAs. In zebrafish embryos, the germ plasm segregates again to the prospective primordial germ cells (PGCs) during the first mitotic divisions. *bucky ball (buc)* is the only known gene whose function is required to establish polarity, specify the first axis, and set aside the germline through an unknown mechanism and pathway. We identified RNA binding protein with multiple splicing 2 (Rbpms2) as a Buc binding partner. Rbpms2 is implicated in binding RNAs that encode proteins involved in regulating germ line development. Rbpms2 has an RNA recognition motif and no other structurally recognizable domains.

To investigate the spatial and temporal distribution of Rbpms2 protein we generated a GFP-Rbpms2 fusion protein. In transient expression assays, we microinjected mRNA encoding GFP-Rbpms2 and found that the fusion protein becomes restricted to the PGCs. While the 3'UTR plays a prominent role in regulating localization and translation of mRNAs, our *gfp-rbpms2* construct lacks the 3'UTR; therefore, it must accumulate in PGCs through a different mechanism. Here we used a structure-function approach to identify regions of Rbpms2 protein necessary and sufficient for PGC localization in zebrafish.

Nanoparticle Contrast Agent for Multi-modality Molecular Imaging and Cancer Theranostics

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Tumor metastases are a major source of pain in cancer patients and are difficult to detect and treat, even with the most advanced of imaging devices. We thus aim to enhance detection and improve methods of delivering therapeutics to metastatic tumors. Using animal models of human cancers, our studies focus on the utilization of nanoparticle agents to serve as contrast agents for imaging technologies. Nude mice were injected with human cancer cells. Tumors developed within 2-3 weeks, were detected with bioluminescent imaging, and were subsequently imaged by micro-positron emission tomography (PET), computed tomography (CT), and magnetic resonance imaging (MRI). Gadolinium oxide nanoparticles, provided by Mahantesh Navati, PhD, were tested as contrast agents. In pilot studies, nanoparticles were then tested for their ability to serve as potential drug delivery agents, using the drug adriamycin. Nanoparticles were localized using an external magnet. Increased glucose uptake within tumors was observed when the mice were injected with 18F-FDG and imaged with Micro-PET. Enhanced contrast intensity was observed in MRI after nanoparticle magnet treatment. When the adriamycin drug was attached to the nanoparticles in pilot studies, bioluminescent imaging suggested that tumor sizes significantly decreased over a period of 5 days. This implicates the successful use of gadolinium oxide nanoparticles as a chemo-theranostic agent. We aim to perform further studies using other drugs attached to the nanoparticles to further understand this potential advancement in cancer theranostics.

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Genetic analysis of homomeric and heteromeric interactions of HIV-1 integrase with the host factor INI1/hSNF5

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HIV-1 Integrase (IN) is a virally encoded enzyme that catalyzes the integration of viral DNA into host genome. IN exhibits both homomeric and heteromeric protein-protein interactions. INI1/hSNF5 is a host protein that directly binds to HIV-1 IN. It is a core component of the ATP-dependent chromatin-remodeling complex, SWI/SNF and also a tumor suppressor. INI1 is required for HIV-1 particle production, is encapsidated into HIV-1 virions and is required for infection of particles. Previously, a yeast reverse two-hybrid system was used to isolate INI1-interaction-defective (IID)-IN mutants. These IID-IN mutants, such as D202G, were severely impaired for replication.

A yeast two-hybrid system was used to characterize homomeric interactions of D202G mutant and to isolate compensatory mutations in INI1 that restores the interaction with D202G. To determine the homomeric interactions, plasmids encoding GAL4-Activation domain (GAL4-AD) fused to IN (WT or D202G) were co-transformed with plasmids encoding LexA DNA binding domain (LexA-DB), also fused to IN (D202G or WT) into yeast. The interaction between IN-IN, IN-D202G and D202G-D202G were assessed based on their ability to induce the *LacZ* reporter gene expression using an X-Gal assay. We found that D202G IN retained homomeric interactions indicating that it is specifically defective for interaction with INI1.

To screen for mutants of INI1 that acquired the ability to bind to D202G, a random INI1 point mutation library (GAL4-AD-IN*) was co-transformed into yeast along with LexADB-D202G. Initial screening of 30,000-40,000 yeast transformants has yielded several blue colonies. We are in the process of isolating and sequencing the plasmids from these colonies to determine the nature of mutations that may have conferred INI1 ability to bind to D202G. Characterization of these compensatory mutations are likely to yield valuable structural information and may lead to rational drug design to combat HIV-1.

Multisensory Processing In Children With Autism

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Viewing a person's articulatory movements substantially improves a listener's ability to understand spoken words, especially under noisy environmental conditions. A prominent theory in autism proposes that automatic multisensory integration (MSI) is impaired in this population, thereby inhibiting effective perception. However, direct empirical support of such deficits remains scarce. Impairment in communication is one of the hallmark symptoms in autism and the ability to perceive speech is a fundamental prerequisite for communication.

In our study, we assessed whether the integration of auditory and visual speech signals is impaired in high functioning children with ASD, by presenting them with monosyllabic words in auditory alone, audiovisual (AV) and visual (V) alone conditions, under varying signal-to-noise ratios. If MSI is indeed impaired in persons with autism, results signifying reduced gain in AV Integration would be expected. A large deficit in the ability of ASD children (ages 7-12) to integrate information from two senses was indeed expressed, as reduced AV gain, while performance in the auditory alone conditions was relatively normal. However, surprisingly, ASD children, ages 13-17, exhibited comparable AV gain with TD teens, implying a recovery of MSI in the teenage years. This finding provides hope for parents of ASD children that, assuming no mechanism is inherently broken, early intervention may drastically reduce the MSI deficit exhibited by younger ASD children. Differences in how multisensory inputs are integrated, and how these differences affect higher-order processing, as well as the impact of early intervention on the pathogenesis of persons with ASD, remain to be explored.

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Associations Between *CRKL*, *UFD1L*, and *VEGFA* and Clinical Variability of Heart Defects in 22q11.2 Deletion Syndrome

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The 22q11.2 deletion syndrome (22q11DS), also known as velo-cardio-facial/DiGeorge syndrome, is caused by a hemizygous microdeletion on chromosome 22. The syndrome is characterized by cardiac outflow tract malformations and craniofacial, parathyroid, and thymic defects, with most patients showing significant clinical variability. Animal studies have identified several candidate genes which contribute to these phenotypes, including *TBX1* and *CRKL*. There are also additional candidate genes on the 22q11.2 region (*UFD1L*) and elsewhere (*VEGFA* on chromosome 6).

We hypothesized that variations of single-nucleotide polymorphisms (SNPs) within the candidate genes affect the severity of the heart defects in 22q11DS patients. The lab tested *TBX1* but found no significant correlations between the severity of heart defects and common SNPs in the gene. Using the National Center for Biotechnology Information SNP database, we selected 13 SNPs from candidate genes—8 from *CRKL*, 3 from *VEGFA*, and 2 from *UFD1L*—along with 20 other SNPs from various genes within the deletion region and genotyped them in our 22q11DS cohort with the Sequenom MassARRAY iPlex platform. From preliminary data, we found a significant correlation between the SNP rs756875, which is located downstream of *CRKL*, and the presence of severe heart defects including tetralogy of Fallot, persistent truncus arteriosus, and interrupted aortic arch. The remaining assays are in process and the genotype data should be available within the next few weeks. This may provide a better understanding of genotype-phenotype correlations and the vast range of phenotype severity seen throughout this syndrome.

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Kinetic Properties of the *Mycobacterium tuberculosis* Enzyme Isocitrate Lyase

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The glyoxylate shunt is a metabolic pathway that catalyzes the transformation of isocitrate to succinate and glyoxylate via isocitrate lyase (ICL) and glyoxylate and acetyl coenzymeA into succinate and malate via malate synthase (MS). The glyoxylate shunt is essential for growth on two carbon sources such as fatty acids, which are a major source of energy for *Mycobacterium tuberculosis* in infected tissue . Thus, in *M. tuberculosis* it has been shown to be essential for persistence and survival in host tissue, especially in activated macrophages. While the glyoxylate pathway has been observed in all three kingdoms, it has never been observed in humans. This, coupled with the fact that the glyoxylate pathway is necessary for *M. tuberculosis* persistence in human hosts makes the enzymes involved an attractive target for potential drug therapies. This study focuses on isocitrate lyase, the first enzyme in the glyoxylate pathway which catalyzes the cleavage of isocitrate to form succinate and glyoxylate, as well as the reverse reaction. The aim of this research is to characterize the kinetics of the reactions catalyzed by ICL. By doing this, more effective treatments can be developed to specifically target and inactivate them, enhancing our ability to treat the disease.

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Elucidating the mechanism for αA-Crystallin transcriptional regulation at the earliest stage of lens development

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The ocular lens is used to study cell lineage and terminal differentiation because of its simplicity. All cells can be traced to the lens placode. Several things characterize lens differentiation. Progenitor cells i.e. the lens vesicle separates from the ectoderm. The cells in front remain epithelial, while posterior cells become primary lens fibers.

Lens differentiation is marked by increasing expression of the αA- crystallin gene. Crystallins are water-soluble proteins that impart transparency and refracting abilities. The enhancers DCR1 and DCR3 control gene expression[1]. However, expression of the EGFP gene driven by DCR1 or DCR3 does not commence during lens pit formation (around E10.5). In contrast, a 15 kb fragment containing the 11 kb of the 5'-flanking region including DCR1 and lacking DCR3 is expressed at E10.5 in transgenic mice[2]. The histone H3K4 me1 profile along the Cryaa locus revealed a peak 3' from DCR1 and in a region ~4 kb upstream from the promoter[3]. This denotes tissue-specific enhancers. We hypothesize that a 3'-region flanking DCR1 serves, with DCR1, as the earliest Cryaa enhancer. As a test, DCR1 was deleted from the 15 kb fragment and additional transgenic reporters were generated.

Previously, a deletion of DCR1 and a larger deletion was made to generate an 11 kb Cryaa/EGFP[2]. There was no expression of EGFP at E10.5 in multiple transgenic lines. DCR1 is essential for expression but is not sufficient in the lens pit. Four EGFP reporter constructs were prepared via PCR and subcloning into the p1.9 kb Cryaa/EGFP vector. They will be used to generate novel transgenic mouse lines. If we are correct, the 2 kb DCR1 will be sufficient for expression at E10.5.

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Iron Oxide Nanoparticles for Multifunctional Biomedical Applications

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Magnetic nanoparticles offer numerous applications due to their versatility in all areas of medical treatment and diagnosis. Utilizing their magnetic properties, nanoparticles can be used for biomedical processes such as enhancing molecular imaging, improving current drug delivery systems, and increasing the efficacy of therapeutic drugs while decreasing side effects.

In this project, iron oxide nanoparticle development, stabilization, binding affinity, and drug release profile were studied. Iron oxide nanoparticles are of great interest due to their potential to have super-paramagnetic properties. However, it is very difficult to consistently produce monodisperse nanoparticles. The formation of iron oxide nanoparticles through co-precipitation is influenced by temperature, concentration, and most importantly, the pH of the solution at equilibrium. Thus, identifying the optimal pH was our primary focus during development. Particle size was determined through Dynamic Light Scattering (DLS) analysis.

Once a consistent protocol was developed with an optimal pH, the resultant particles were coated with oleic acid, followed by coating of various different drugs in order to test and compare the results for such applications as MRI enhancement and anti-cancer treatment in mice. Due to time constraints, extensive analysis of only Adriamycin coated nanoparticles could be performed. Adriamycin is drug used in chemotherapy treatments. The binding affinity and drug release profile was analyzed via a fluorescence machine. It was found that Adriamycin has a high binding affinity to the iron oxide nanoparticle oleic acid complex, and the rate of release increases linearly with pH. These promising results give rise to a new level of treatment and diagnosis in biomedicine, as it can be applied to a variety of different drugs.

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Myc function in genome rearrangements and aging

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Myc serves many well characterized functions in the cell, including regulation of proliferation, cell growth and apoptosis. It is also a proto-oncogene found to be misexpressed in a large number of human cancers. Myc functions by binding to the protein Max to form heterodimers that bind to E box DNA sequence (CACGTG) to activate transcription. *Drosophila* fitted our studies since the function of Myc in mammalian and *Drosophila* is highly conserved.

My project focuses on the role of Myc in the induction of genomic instability and sought out to determine if dMyc overexpression in adults increased mutation frequency, and if dMyc overexpression specifically in CNS decreased *Drosophila* adult lifespan.

Both aims were approached with the UAS/Gal4 system to overexpress dMyc. We assessed mutation frequency in Drosophila using a LacZ reporter system. These flies were allowed four days of overexpression. Then LacZ removed, plasmid ligated, and transformed into *E. coli* for calculations of mutation frequency. For the longevity assay, the transgenic flies and WT were kept at 29ºC and every 2-3 days casualties recorded.

We concluded that when dMyc overexpression is restricted to cells in the CNS, lifespan is not as dramatically altered. The transgenic flies do exhibit to be short lived and begin their decline faster than WT. The mutation frequency was no able to be calculated due to lack of time.

None of this could have been achieved without the help from the Secombe laboratory and funding from the Department of Genetics AECOM, and SURP.

The Role of Nkcc1 in Maintaining Cortical Integrity

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Nkcc1 is highly expressed during late stages of development and early postnatal stages. Its peak expression during this developmental period renders GABA (a normally inhibitory neurotransmitter in adult animals and humans) excitatory. However without the role of Nkcc1, neurons are unable to form proper synapses, and Nkcc1 knockout mice exhibit a unique phenotype of motor hyperactivity that is often seen in the schizophrenic mouse model. Based on the motor hyperactive symptoms of the mice, as well as the known associations with the prefrontal region in schizophrenia, cortical tissue was chosen to be analyzed. Western Blots were performed with homogenized brain tissue of Nkcc1 mutants and wild type littermates to look for molecular differences in the cortex. In particular, receptors associated with NMDA, glutamate, and dopamine was analyzed. A separate group of mice received injections of haloperidol, a dopamine antagonist used to treat schizophrenia which alleviates the hyperactive symptoms of the Nkcc1 mutants, and their brain tissue was analyzed for changes in the same receptors as done previously. Statistical significance in the difference between wild-type and mutants was found on a .05 level with the protein Tyrosine Hydroxylase (TH), which is associated with dopamine signaling. Nkcc1 mutant mice remain a good potential model for schizophrenia and its role in promoting these abnormal phenotypes should be further studied.

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Hydroxyl Radical Footprinting of RNA by Pyrite

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Knowledge of the solvent accessibility of a biopolymer assists in understanding its conformation and its function. Solvent accessibility of RNA changes by both, protein binding and 3-dimensional structure formation. Hydoxyl radicals are suitable footprinting tools to determine the solvent accessibility of biopolymers due to their high reactivity and small size. A microfluidic device utilizing pyrite (iron sulfide) has been described as an alternative to the traditional Fenton (Fe(II)-EDTA) based hydroxyl radical footprinting of DNA, with the potential for high-throughput applications. In order to test if RNA can be cleaved as well, pyrite footprinting experiments were conducted on the 49-mer Diels-Alderase (DA) ribozyme using a pyrite matrix (63-180 μm) in a cartridge. The 5'-terminus of the DA was ^{32}P-labeled for visualization of the fragments on a 15% polyacrylamide gel with as good as single nucleotide resolution. The flow rate of the sample through the device was varied in order to optimize the experimental procedure to ensure single hit kinetics. Ion concentrations of mono- and divalent cations were varied to evaluate their effect on RNA absorbance to the pyrite matrix and the production of hydroxyl radicals. RNA fragments were quantified by analysis software. Single hit kinetics was maintained at flow rates of 80-100 uL/min and 1% hydrogen peroxide. Variation in cation concentration indicated that there was no significant RNA absorbance at any of the ion concentrations tested as the CPM recovery was consistently close to 80%. Comparison of the cleavage profiles between pyrite and the Fenton based hydroxyl radical footprints showed that the two methodologies were equivalent. Pyrite footprinting using mini- and micro fluidics will now be used to analyze larger RNA molecules such as the *Tetrahymena* ribozyme.

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Eicosanoids and Chagas Disease

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Trypanosoma cruzi, the etiological agent of Chagas disease, causes vasculopathy and cardiomyopathy in humans and is associated with elevated levels of several vasoactive molecules such as nitric oxide, endothelin-1, and thromboxane A_2 (TXA_2). Our laboratory has demonstrated that *T. cruzi* is capable of synthesizing TXA_2 and that TXA_2 signaling controls pathophysiology of Chagas disease. TXA_2 receptor (TP) null mice and infected mice treated with aspirin (ASA) displayed increased parasitemia, mortality, and cardiac pathology compared with wild type (WT) and TXA_2 synthase (TXA_2S) null mice. In this study, we evaluated the extent of the arachidonic acid (AA) metabolic pathway in the parasites and found that the parasites express a protein reactive to human cyclooxygenase (COX) and phospholipase A_2 (PLA_2) antibody. We also investigated the role of TXA_2S signaling *in vivo* employing TXA_2S, TP, and COX-1 null mice and compared parasite-induced inflammation in different organs both at the acute stage (30 dpi) and chronic stage (60 dpi) of infection. Inflammation in TP null mice was greater than the WT and other null mice types studied. Interestingly, the pancreas and urinary bladder of infected mice showed intense inflammation in all the mice studied. We further evaluated infection-induced expression of COX-1, COX-2, 5-LOX, 12/15-LOX, and SOCS-2 in different organs of infected mice through immunoblotting.

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Functional analysis of Memory CD8+ T-cells ex vivo

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Vaccines usually confer improved protection against microbial diseases through the formation of long-lived memory cells in vaccinated individuals. Memory cells are lymphocytes. While B lymphocytes mediate humoral immunity (antibodies), T lymphocytes mediate cellular immunity. In turn, they are subdivided into CD4/helper and CD8/cytolytic T lymphocytes. The naïve lymphocytes present in the host undergo initial activation by recognizing their cognate antigen and further differentiate into memory cells that are long-lived and exhibit improved functional features that mediate most efficient protection of immunized hosts. In depth understanding of the mechanism regulating such improved ability to protect is lacking. My SURP project was to investigate the functional characteristics of protective memory CD8+ T-cells ex vivo. I studied these events in mice immunized with the intracellular bacterium *Listeria monocytogenes* (LM) which developed memory CD8+ T-cells that confer life-long protection to the host against a secondary infection with an otherwise lethal dose of bacteria. Memory CD8+ T-cells taken from wt Lm immunized mice responded to a lower concentration of their cognate antigen and therefore are more sensitive. In addition, memory cells from wt LM immunized mice produced high levels of CCL3 and TNF cytokines. However, when transcription and translation were inhibited respectively, LLO memory CD8+ T-cells showed the most inhibition. Memory CD8+ T-cells from mice immunized with Wt LM OVA were the most widely distributed and exhibited the greatest functional quality.

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“Towards High Throughput Footprinting of DNA by Iron (II) Sulfide”

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Information on the conformation of DNA is invaluable when studying macromolecule interactions. Highly reactive hydroxyl radicals (·OH) can be used to assess the solvent accessible surface areas of nucleic acids. ‘Hydroxyl radical footprinting’ experiments reveal structural information with single nucleotide resolution. Many methods, including synchrotron irradiation and Fenton chemistry, are able to generate ·OH radicals; however, they are not compatible with high throughput structure determination of nucleic acids by footprinting. Recently, iron (II) sulfide (pyrite) embedded in a microfluidic device was shown to serve as a source for OH radicals to footprint radiolabeled double stranded DNA. The goals of this study are: i) to test the activity of a pyrite filled mini cartridge; and ii) to establish a protocol which allows the use of ^{32}P and fluorescently labeled primers to detect cDNA fragments by traditional sequencing and by capillary electrophoresis.

Radiolabeled 54nt and 96nt dsDNA sequences were flowed through the pyrite mini cartridge under oxidative and peroxidative conditions. Enhanced cleavage was detected in presence of 1% H_2O_2 while the absence of hydrogen peroxide did not lead to significant fragmentation under the same testing conditions. To increase throughput, non-radioactively labeled DNA fragments generated by the Fenton Reaction and the pyrite cartridge were extended with fluorescently labeled and, in parallel, with ^{32}P labeled DNA primers. In both cases, dose response experiments showed that less full length cDNA is detected with increasing hydroxyl radical concentration. However, no significant amount of fragments could be detected. Further research will focus on optimizing the radioactive technique to customize it for fluorescence detection. Once the fluorescence method is perfected, further research will focus on quantifying the fragmentation pattern with increasing rounds of primer extension.

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High Throughput Screen for Equilibrative Nucleoside Transport Inhibitors: Search for an Antimalarial Drug

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Malaria is a serious world health concern. It is one of the most costly diseases known, with over 300 million cases diagnosed worldwide each year resulting in over one million deaths. Infection by *Plasmodium falciparum*, an intracellular parasite, is responsible for the most severe cases of malaria. It may cause death if not treated with 24 hours of the onset of symptoms. *P. falciparum* is particularly problematic as it has developed resistance to some of the commonly used antimalarial drugs, including chloroquine.

The Plasmodium falciparum Equilibrative Nucleoside Transporter 1 (PfENT1) is a potential target for antimalarial drug development. *P. falciparum* is a purine auxotroph, thus, it must import purines from the host. PfENT1 is the main purine transporter during the pathogenic intraerythrocytic stages because PfENT1 knockout parasites cannot survive at physiological purine concentrations (El Bissati et al.).

To find PfENT1 inhibitors we are designing a high-throughput assay to screen large chemical compound libraries. We have successfully expressed PfENT1 in a *C. aethiops* kidney cell line (COS1), which has a pharmacologically inhibitable endogenous nucleoside transporter. In addition, we have successfully cloned PfENT1 into plasmids which will allow us to express PfENT1 in an *S. cerevisiae* yeast strain (FUI1Δ) that lacks the endogenous nucleoside transporter. We will test cell viability in the presence of the cytotoxic adenosine analogue tubercidin, which is transported by PfENT1 (Riegelhaupt et al.). In our assay, if PfENT1 has been successfully inhibited, we would expect to see viable cells.

We hope that our assay will allow us to discover possible drugs that block PfENT1, and thus would help treat malaria.

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Towards revealing the structure of the IMP 2,3 RNA recognition motifs

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RNA binding proteins bind mRNA transcripts and regulate their nuclear export, localization, stability and translation. Insulin-like growth factor II messenger RNA binding proteins (IMPs) are a highly conserved family of developmentally essential oncofetal RNA-binding proteins shown to regulate RNA metabolism. IMP2 and IMP3 have proven to be reliable diagnostic biomarkers for diabetes mellitus and multiple neoplastic conditions. IMPs share a characteristic arrangement of six canonical RNA-binding domains, consisting of two RNA recognition motifs (RRM) followed by four hnRNP-K homology (KH) domains. Recent studies have provided insight into the function of the KH domains of IMP1 in RNA localization and translation. However, the specific contribution of the N-terminal RRM domains of this family of RNA-binding proteins toward RNA metabolism remains uncertain. It is our hypothesis that knowledge of the precise structural arrangement of the IMP RRM RNA-binding domains is required for understanding how IMPs regulate mRNA. Here we present methods to express, purify, and crystallize the RRM12 domains of IMP1-3 for further structural and biochemical analyses.

We expressed and purified the RRM12 domains of IMP1-3 by affinity chromatography using an N-terminal maltose binding protein (MBP) tag and C-terminal His-6 tag. A TEV protease cleavage site was utilized to separate the MBP tag from IMP1-3 RRM12 after purification. IMP2 RRM12 and IMP3 RRM12 constructs demonstrated adequate purity and solubility. Pure protein was then concentrated volumetrically 15-20x with a centrifugal filter tube. Concentration was assessed by spectrophotometry. Crystallization trials of IMP2/3 RRM12 are ongoing.

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