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2010 ABSTRACTS

Summer Undergraduate Research Program

Graduate Programs in the Biomedical Sciences
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ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY

GRADUATE DIVISION OF BIOMEDICAL SCIENCES

SUMMER UNDERGRADUATE RESEARCH PROGRAM

2010

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Assistant Dean for Graduate Studies
Director, Summer Undergraduate Research Program

2010 SUMMER UNDERGRADUATE RESEARCH PROGRAM

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Senescence, a Surrogate Marker of Differentiation, Predicts Outcome to Taxol Therapy in Breast Cancer

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Tumors are highly heterogeneous, which is a major cause of resistance to chemotherapy drugs, such as Taxol. Taxol binds to cellular microtubules and suppresses mitosis. Consequently, cells accumulate damage and cannot resume normal proliferation. Two mechanisms exist in damaged cells to avoid replication. The first is senescence, whereby cells are "irreversibly" arrested and fail to re-enter the cell cycle after mitogenic stimulation; or cell death, of which apoptosis is the best-described.

Cells that are terminally differentiated (e.g. neurons) cannot become senescent and instead undergo cell death. It is widely assumed that the prevalent chemotherapeutic response in patients is cell death and that this is the major mechanism of tumor regression. However, since cancer cells have various degrees of differentiation, we hypothesized that the mode of proliferative arrest (apoptosis versus senescence) depends on the intrinsic differentiation status of a cell and / or tumor.

To address this, we quantified senescence in breast cancer cell lines before and after treatment with Taxol and correlated the mode of proliferative arrest with markers of (1) differentiation (CD44 / CD24 status) and (2) Epithelial to Mesenchymal Transition, EMT, (miR-200c, vimentin and E-cadherin). Two classes of breast cancer cells were identified. The first had high levels of both basal and Taxol-induced senescence, were ER+ and heterogeneous for CD44/24 expression (MCF7 and T47D). The second class was ER-, homogenous for CD44/24 expression, had low basal senescence and underwent cell death after Taxol treatment rather than senescence (MDA-MB-231 and MDA-MB-468). Interestingly, this class corresponds to basal-like breast tumors that respond well to therapy but have a high frequency of relapse and high proliferation indices. Markers of EMT did not correlate with senescence and miR200c expression, a marker of metastasis, only correlated with CD24 status. These findings suggest that senescence is a reliable marker of differentiation potential that dramatically influences the mode of proliferative arrest (death versus senescence) in response to chemotherapy-induced cell damage.

The Effects of CSF-1R Mutation on Macrophage Motility and Functionality

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Macrophages play important roles in normal tissue development and immune function. They perform homeostatic functions such as phagocytosis of apoptotic cells and pathogens. However, macrophages are also involved in the pathogenesis of many diseases, including arthritis, atherosclerosis and metastatic cancer. For this reason, macrophages have become the focus of much scientific scrutiny.

Colony-stimulating factor 1 (CSF-1) plays important roles in macrophage proliferation, differentiation, and motility by binding and signaling via the CSF-1 receptor tyrosine kinase (CSF-1R). The CSF-1R has seven tyrosine residues that are known to be phosphorylated following CSF-1 binding, several of which have been implicated in macrophage function. Here we examine the role of Y721 and Y706 in macrophage function compared to the wild-type receptor (WT) and to a minimal functional receptor that allows cell survival (DAB). To analyze each residue's importance in motility, a CSF-1 upshift assay was performed. The Y721F, Y706F, and DAB mutants all demonstrated significantly reduced ruffles than the WT. The ability of each mutant to internalize particles, by a process known as phagocytosis, was then investigated. While not statistically significantly different, a clear trend existed with the WT performing better than any of the mutants. Additionally, the ability of the mutants to migrate across an endothelial barrier was analyzed; results indicated that DAB migrated independent of tumor necrosis factor alpha (TNFα). This may be due the level of differentiation of the different cell lines and suggested that the differentiation state may be playing a role in transendothelial migration. To test this hypothesis, DAB cells were stimulated with phorbol 12-myristate 13-acetate (PMA), a well-characterized inducer of differentiation. PMA-treated DAB macrophages were found to act more like the WT, though further analysis is needed to verify this. These experiments suggest that Y721 and Y706 are required for CSF-1 induced macrophage function, and that the differentiation state of each mutant may be playing a significant role in their function.

Acknowledgements: Authors would like to thank the 2010 Summer Undergraduate Research Program (SURP) at the Albert Einstein College of Medicine. This work was supported by NIH grant R01 GM071828 (DC)

Developmental sex dichotomy of the connexin43-dependent transcriptomic networks in the mouse heart

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Connexin43 (Cx43), the most abundant gap junction protein in mammals, has been shown to be critical for proper heart function: Cx43 null mice die shortly after birth due to right ventricular outflow tract obstruction. The Iacobas lab found that *Gja1*, the connexin43 gene, is a sort of 'hub' that modulates the heart rhythm determinant (HRD) gene fabric. In order to elucidate the developmental sex dichotomy of the Cx43-dependent transcriptomic networks of the heart, we profiled the transcriptomes of the atria and ventricles of males and females 19 days embryonic, 7 day-old and 14 day-old wildtype and embryonic Cx43 KO mice using Illumina BeadChips. In contrast to post-pubescent heart findings, there was a lack of differential expression between sexes among the HRD genes at the younger stages, lending to the theory that sex-dependent cardiac phenotypes result from pubertal changes rather than intrinsic genetics. Differentially expressed genes were clustered using the DAVID Functional Annotation Tool, revealing that transcriptomic differences of different gene clusters develop between sexes during maturation. Moreover, the patterns of gene cluster expression were modified by disruption of Cx43 expression in the embryonic heart. These included alterations in clusters associated with heart morphogenesis and neural crest cell development, processes strongly associated with the structural abnormalities observed in Cx43 null mice. Since most of the effort went toward gathering, organizing, and processing the data, there remains an enormous potential for further analysis. Thanks to the Albert Einstein SURP program for funding and the Iacobas Lab for being wonderfully helpful and patient.

Binding of fungal melanins by anti-melanin antibody for the purpose of radioimmunotherapy

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Cryptococcus neoformans and other fungal pathogens such as *Wangiella dermatitidis* represent a significant health risk to immunocompromised patients around the world. In the case of AIDS, cancer, and organ transplant patients, all of whom have suppressed immune systems; these fungal infections can spread to the central nervous system and cause fungal meningitis which is often fatal.

The ability of microbe-specific antibodies to localize ionizing radiation to the sites of fungal infection has been first demonstrated by our laboratory (Dadachova E. et al. PNAS 2003). Fungal melanins are found in the cell wall of all major human pathogenic fungi. Thus, we hypothesize that the antibody to fungal melanin could deliver cytocidal doses of ionizing radiation to various melanin containing fungal pathogens, thereby eliminating the need for developing a treatment for every particular fungus.

Binding of 6D2 was demonstrated visually through the use of immunofluorescence. Cells were dried on slides and then incubated with the murine 6D2 anti-melanin antibody, followed by a second incubation with goat anti-mouse FITC conjugated antibody. The fluorescent FITC secondary antibody made it possible to determine if 6D2 had bound specifically to the fungal cells.

The fluorescent images of the lightly melanized fungi provide evidence of specific binding by the 6D2 antibody. Both the highly melanized and non-melanized fungi revealed no binding by the anti-melanin 6D2 antibody. Fungal melanins form shell like, layered structures and after heavy melanization it is possible that some of the epitopes recognized by 6D2 are not accessible for binding.

Acknowledgements

Special thanks to Dr. Ekaterina Dadachova (supported by NIH grant AI60507), Dr. Ruth Bryan, and Dr. Zewei Jiang for giving me the opportunity to work with them. Thank you to Dr. Josh Nosanchuk for his advice regarding IF. Also thank you to Rosa Garcia, Victoria Freedman, Nilda Soto, and SURP for organizing the summer program.

Comparison of six capsid proteins to TRIM5α-Rh-Flag to determine Protein-mediated restriction of HIV-1

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Human immunodeficiency virus (HIV-1) is blocked in the cells of most Old World monkeys due to evolutionary mutations of the retroviral restriction factor TRIM5α. The determinant for TRIM5α-mediated restriction is the capsid protein which assembles into the shell that protects the nucleic acid of the Virus. The restriction mechanism is unknown; however, the TRIM5α B30.2 domain may interact with the capsid of the incoming virus, leading to a destruction of the infection process. We can use the information known about TRIM5α to determine if other proteins also restrict HIV-1. Our goal is to determine if capsid binding interaction is present for six unknown proteins. First the proteins were Maxi-prepped to extract the plasmid DNA, along with TRIM5α–Rh-Flag which was used as a control. 293FT cells were transfected with these plasmids to express the different proteins and determine the concentration of the samples by Western Blotting. Using these concentrations, 293FT cells were transfected with 10μg of each plasmid DNA to perform capsid binding to HIV-1 CA-NC complexes. Lastly the level of interaction with the capsid was determined by Western Blot. It was concluded that the protein CKAP1 restricts Human immunodeficiency virus (HIV-1) and MAP1A, MAP1S, and ImpB3 do not. Results for hrRNPI and WIRE1 proteins were inconclusive. In the future we will attempt the experiment again with different concentrations and better conditions to get more accurate results.

Expression Profile Changes During the Differentiation of Human Mesenchymal Stem Cells into Osteoblasts

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Osteosarcoma is the most common primary bone tumor, with approximately 400 cases reported annually in the United States. The cell of origin of osteosarcoma remains unclear. Human mesenchymal stem cells (hMSCs) are capable of differentiating into osteoblasts, chondroblasts, and fibroblasts, as well as other cell types. We hypothesize that the cell of origin of osteosarcoma lies on the differentiation continuum between hMSC and osteoblast. Normal hMSCs were induced to differentiate into osteoblasts. Microarray analyses were performed to determine the expression profiles of the hMSCs and osteoblasts, with the intent to compare these profiles to those of osteosarcoma. Flow cytometry confirmed the presence of hMSCs. Alizarin Red S staining indicated that differentiation into osteoblasts had occurred, and enzyme immunoassay confirmed this impression. Microarrays were performed, and data analysis is pending; as a result, the genes that undergo expression changes during differentiation have not yet been determined. Future experiments will include surface marker analyses at intervals throughout hMSC differentiation into osteoblasts, as well as microarray analyses of hMSC differentiation into cell types other than osteoblasts. Elucidation of the cell(s) of origin of osteosarcoma could have significant clinical implications.

Acknowledgements: Dr. Gorlick and his Lab Staff, Pediatric Hematology/Oncology Department, Albert Einstein College of Medicine Summer Undergraduate Research Program

Hyper-methylation of Zinc Finger Genes on 19q13 in Oropharyngeal Cancer

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Head and neck cancer is the fifth most common malignancy in men worldwide. The five year survival rate of patients with head and neck squamous cell carcinoma has improved only marginally in the past three or four decades. Epigenetic mechanisms of cancer are increasingly recognized as a major contributing factor in tumor development. Methylation of DNA is an important phenomenon for the proper development and maintenance of cellular differentiation. Aberrant methylation results in the silencing of genes that maintain transcriptional integrity. Our group is interested in understanding epigenetic changes that occur in head and neck cancer. Preliminary studies using genome-wide scans of DNA methylation in oropharyngeal tumors identified a cluster of aberrantly methylated transcription factor genes located on chromosome 19q13. Using Taqman real-time PCR, we show these aberrant methylation events were accompanied by transcriptional down-regulation of three zinc finger genes in primary oropharyngeal tumors: ZNF542, ZNF447, and ZNF132. Our work reports for the first time on the aberrant methylation of these potential tumor suppressor genes in oropharyngeal cancer, and represents a new avenue of exploration for pathways affected in this disease. Future studies using bisulfite sequencing are needed to validate the methylation status of ZNF542, ZNF447, and ZNF132. In addition, cell line experiments examining the phenotypic results of re-expressing each of these genes is needed to gain insight as to possible function of these genes.

I would like to thank the Summer Undergraduate Research Program at the Albert Einstein College of Medicine for a wonderful experience and Dr. Olivier Loudig for his help with the RT PCR machine. This work was supported by grant R21CA131648 (TJB) from the NCI.

Septin 9 Isoform Expression and Function in Breast Cancer Cells

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Septins are a complex family of GTPases containing 14 different members involved in various cellular processes such as vesicle trafficking, apoptosis and interactions with the cytoskeleton. Septin 9 (SEPT9) is of particular interest because of its role in oncogenesis. The complexity of this gene is due to its ability to form up to 18 isoform variants. Our project is to elucidate the specific role that five of these isoforms, each differing in their 5' region, maintain during breast tumorigenesis. We have generated isoform specific GFP constructs in MCF7 breast cancer cells. Analysis of these SEPT9 isoforms has revealed differing morphologies which may signify that each has a specific function during breast cancer. We have observed differences in migration rates and growth patterns among each of the five isoforms, looking at single cells versus clusters, which could be affecting this migration based on their ability to form tight junctions. Proliferation assays confirmed that there was also a difference in the growth rate among the isoforms, with v4 and v1 exhibiting the slowest growth rate, while v4 and v5 display the fastest. Lastly, localization between isoforms was measured using GFP signal intensity. It was found that v1, v3, and v5 localize primarily to the nucleus, indicating that these may have a role in transcription while v2 and v4 localized mainly to the cytoplasm and may be affecting cytoskeletal structure. These observed morphological and functional differences exhibited among the five isoforms support that each retains its own significant role in breast tumorigenesis.

Acknowledgements: Funded by the Albert Einstein College of Medicine Summer Undergraduate Research Program. Special thanks to Dr. Cristina Montagna and the Montagna lab.

Role of the centrosomes on the Localization of Drosophila Kinesin-13, KLP10A to the spindle pole.

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Kinesin-13s comprise a subfamily of microtubule-based motors that perform essential functions throughout the cell cycle. During mitosis, the kinesin-13, KLP10A, localizes to the centrosome and the spindle pole and drives chromosome segregation by depolymerizing pole-associated microtubule minus-ends. While apparently not functional at the centrosome, KLP10A is mislocalized from the pole, its primary site of action, when centrosomes are functionally ablated. Moreover, KLP10A has been observed to shuttle from the centrosome to the spindle pole (unpublished results). These data suggest that centrosomes are a waystation through which KLP10A must pass to become active. To test this hypothesis further, we have investigated whether KLP10A's association with poles is dependent on the distance between the centrosome and the spindle pole. To quantify this, Drosophila S2 cells were immunostained with anti-KLP10A and anti-α-tubulin and imaged using the Perkin Elmer ultraview scanning confocal microscope. The results suggest that the initial distance between the centrosome and the spindle pole, influences the total amount of KLP10A present at the active site.

NGAL (Neutrophil gelatinase associated lipocalin-2) significantly reduces proliferation and induce inflammatory responses in renal mesangial cells of lupus mice

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System Lupus Erythematosus (SLE) is an autoimmune disease in which the immune system mounts attacks against various organ systems in the body. One prominently targeted organ system is the renal system. Detrimental changes in kidney function are seen in 40% of lupus patients, and if left untreated can develop into Chronic Renal Insufficiency, which can be disastrous to the patient. Previous studies from our lab had found a novel biomarker, NGAL, Neutrophil gelatinase-Associated Lipocalin, which indicated significant correlation with progression of Lupus Nephritis. It was of great importance to find out the mechanism of action of this novel biomarker which indicates renal pathology at an early stage of disease. Consequently, in this study, we asked what effects NGAL produces on MRL/*lpr* mesangial cells (lpr-MC). We found that NGAL significantly decreased the proliferation of lpr-MC in a dose dependent manner. Additionally, NGAL induced the proinflammatory chemokine CXCL10/ IP-10 release after stimulation. To test the autocrine effects of IP-10 on lpr-MC, we stimulated these cells with IP-10 and assessed for proliferation and modulation in NGAL-Receptor /24p3R expression. IP-10 was found to cause moderate decrease in proliferation as well as induce significant upregulation of the NGAL receptor/ 24P3R expression at the mRNA levels. Additionally, IP-10 significantly induced the expression of proapoptotic genes, Bax and APAF-1. Future studies include investigation into whether or not an antibody for IP-10 aids in prevention of worsening disease, as well as the our lab's current studies to validate and identify key pathways involving the mechanism of NGAL in SLE progression. These findings will significantly contribute to the understanding of mechanisms involved in pathogenesis of Lupus Nephritis.

Identification of *Mycobacterium tuberculosis* Components that Regulate Macrophage Production of Tumor Necrosis Factor-alpha

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Tuberculosis (TB) is caused by the pathogen *Mycobacterium tuberculosis* (M.tb). M.tb has developed mechanisms that enable evasion of the host immune response. Tumor necrosis factor-a (TNF) is a critical part of the anti-TB immune response. This cytokine has been shown to enhance antigen presentation and apoptosis, two processes that have been shown to augment immune response to M.tb. We hypothesize that M. tb can evade the host immune response by down-regulating host cell TNF production.

We used an *in vitro* genetic screen to identify M.tb genes responsible for down-regulating macrophage TNF production. A J774.16 mouse macrophage clone, designated C10 stably transfected with the TNF promoter-green fluorescence protein (GFP) fusion construct was used to screen an M.tb cosmid library electroporated into the heterologous *M. smegmatis* mc^2155 strain. One hundred and five *M.smegmatis* M.tb-cosmid clones were subjected to three rounds of screening, and four M.tb-cosmid clones that down-regulate TNF production were identified via measurement of GFP expression and quantification of TNF by ELISA of infected macrophage culture supernatants. Subclones of the four cosmid hits were generated and screened using the TNF reporter macrophages. Several subclones were found to down-regulate TNF production using the C10 system. Initial characterization of these TNF down-regulating cosmids clones have identified M.tb genes whose disruption in the virulent H37Rv strain results in deletion mutants that enhance macrophage TNF production relative to wildtype bacilli.

We have initiated studies to study the biology of the TNF-enhancing M.tb. mutants, to evaluate their ability to promote apoptosis and phagosome maturation, and to test their immunogenicity using a mouse model. These studies should shed light on the mechanisms by which M.tb evades the host immune response and may result in the development of novel anti-TB vaccine.

Acknowledgements
This work was supported by the Summer Undergraduate Research Program at the Albert Einstein College of Medicine to DC and by NIH grants P01 AI 063537 (JC, WJ, SP), R01HL071241 (JC), 5T32CA009173-34 (QJ) and Potts Memorial Foundation Grant (QJ) and the Einstein/Montefiore Center for AIDS Research.

The Effects of 3-Hydroxyanthranilic acid (3-HAA) and 5-Aminoimidazole-4-carboxamide-1-beta-4-ribonucleoside (AICAR) on Gas6 Expression in Several Human CNS Cell Lines

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Growth-arrest specific protein 6 (Gas6) is a secreted growth factor that activates the TAM family of receptor tyrosine kinases, Tyro3, Axl, and Mer, and protects against injury and other stressors by signaling via the PI3 kinase/Akt survival pathway. Mouse models of inflammation and demyelination, such as the cuprizone model and experimental autoimmune encephalomyelitis, have demonstrated the importance of Gas6/Axl signaling in oligodendrocyte and neurons. In this pilot study, we tested the effects of two anti-inflammatory/ immunosuppressive agents, 3-hydroxyanthranilic acid (3-HAA) and 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), on Gas6 expression in several human CNS cell lines. 3-HAA treatment of a neuronal/astrocyte co-culture did not change Gas6 mRNA or protein levels. Treatment of the neuroblastoma cell line SH-EP also appeared to minimally change Gas6 protein levels even in the presence of Brefeldin A, a compound which blocks Gas6 secretion. However, Axl was autophosphorylated and hence activated after 24h of 3-HAA treatment. While Gas6 was unchanged following AICAR treatment of a neuronal/astrocyte co-culture for 24h, a time course will be required to determine whether P-AMPK was activated at earlier time points, since increased phosphorylation of AMPK was not detected at 24h or 48h in a neuronal/astrocyte co-culture, an astrocytoma cell line, an oligodendroglioma cell line, or a GnRH-secreting neuronal cell line. Ongoing studies will measure secretion of Gas6 in spent medium by ELISA.

This work was supported by the SURP program (JC) and National Multiple Sclerosis Society grant RG4046 (BSZ).

The Study of B7-Y as a New Member of the B7 Family

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Molecules in the B7 family and their cognate receptors (typically in the CD28 receptor family) constitute important costimulatory and coinhibitory pathways that control and regulate immune responses generated by T lymphocytes. The B7-Y molecule has been identified in our lab as a potential member of the B7 family, but its immunological function and receptor/ligand is currently unknown.

In this study three methods were used to study B7-Y: first, the B7-Y protein was purified using an expression system. A subsequent function study using 3T3 cells (fibroblast cell line) was performed and data incorporating BWZ cells (T cell line) will be compiled to determine if B7-Y is a receptor or ligand. Second, a binding assay was performed to uncover B7-Y receptors/ligands and demonstrated that B7-Y binds to the membranes of T cells and B lymphocytes. The B7-Y receptors/ligands will then be isolated to determine their composition. Third, the binding between B7-Y molecules was observed and the data suggests B7-Y may bind to other B7-Y molecules. This preliminary investigation uncovered several features of B7-Y's molecular interactions, but further research is needed to fully resolve the function of B7-Y.

This project was funded by the Summer Undergraduate Research Program (SURP) at the Albert Einstein College of Medicine. The authors would like to thank all of the members of the Zang Lab.

Techniques for Studying Myosin IIA Heavy Chain Phosphorylation

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Two characteristics of metastatic cancer cells are their loss of cell-cell adhesion and their ability to invade surrounding host tissue. One of the key proteins in cells that regulate motility and adhesion is nonmuscle myosin-IIA (MIIA). Previous studies from our lab have shown that phosphorylation of the MIIA heavy chain on S1943 leads to filament disassembly and enhanced cell migration. We have developed new tools to study MIIA heavy chain phosphorylation and learn more about the cell signaling pathways that regulate this protein in breast cancer cells. Using newly developed pS1943 MIIA antibodies, in –cell westerns (ICW) are being used to examine MIIA heavy chain phosphorylation in response to a number of different stimuli. To develop a robust in-cell western, first we determined the number of cells needed per well to be in the linear range of detection with these antibodies. To evaluate antibody specificity, we performed peptide blocking experiments and pretreated cells with alkaline phosphatase. Pretreatment of the antibody with a MIIA pSer1943 peptide and pretreatment of cells with phosphatase diminished the antibody signal to approximately background levels. In conjunction with antibody-based studies, we are also examining the functional effects of MIIA heavy chain phosphorylation in breast cancer cells using expression of MIIA phosphomimetics and non-phosphorylatable analogs. However, this has been difficult as MIIA overexpression results in aggregation of the protein. Therefore, we are testing two different promoters that drive expression of a GFP-tagged MIIA to determine whether we can obtain correct protein localization in MDA-MB-231 cells. The CMV promoter allowed for high protein expression levels, which resulted in perinuclear aggregation. The second promoter, a TET-off system containing a TRE response element upstream of a mini-CMV promoter, is currently being investigated for effects on MIIA protein expression. Development of these techniques will be used to further our knowledge of cancer invasion and metastasis, through the understanding of MIIA phosphorylation, and possibly can aid in the detection and treatment of cancers.

Acknowledgements:
Authors thank the Summer Undergraduate Research Program at Albert Einstein College of Medicine, the entire Bresnick lab, and Dr. Karl Wilson of SUNY Binghamton.

Solution NMR Study of Mycobacteria Tuberculosis ATP Synthase Subunit C

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The F_1F_o ATP synthase enzyme is essential for the production of ATP, the main source of energy in almost all biological systems. The enzyme consists of a soluble F_1 portion whose structure is understood and a transmembrane F_o portion for which there is very little structural information. An electrochemical potential drives translocation across the membrane using two transmembrane F_o subunits - a single subunit *a* and a ring of 10-15 *c* subunits. The type of electrochemical potential and number of *c* subunits vary between different species but in the case of Mycobacteria Tuberculosis, the enzyme uses a proton gradient and there are 10 *c* subunits. A recent class of drugs against Tuberculosis, the diarylquinolines, are known to inhibit the *c* subunit causing a significant decrease in intracellular ATP.

Currently, there are two proposed models for the mechanism by which the *c* subunit ring rotates. One involves biased diffusion, wherein protons enter through a half channel in subunit *a*, which abuts subunit *c* in the membrane. Protonation then occurs at a single membrane-buried acidic residue of *c*, neutralizing its negative charge and thus allowing it to rotate into the low dielectric membrane environment. The other proposed mechanism also involves the protonation of an acidic residue in the *c* subunit, but here the protonation induces a conformational change within the subunit *c* monomers, and this structural change drives the rotation of the *c* subunit ring. The purpose of this study was to distinguish between these two models in Mycobacteria Tuberculosis by using solution NMR to probe for protonation-induced conformational changes within the *c* subunit which was reconstituted into detergent micelles. The pH dependent conformational changes in the wild type subunit were compared to those in an altered version of the subunit where the critical membrane-buried glutamate side chain is replaced by the non-protonated glutamine side chain via an E61Q mutation. There was very little change in chemical shift of the peak corresponding to residue D28. It's thought that there a long-range conformational change causes this change but further studies are needed to confirm this hypothesis. The biggest difference in the scaled change in chemical shifts between the E61Q mutant and wild type was found most prominent from residues 50-63. This suggested that there is some sort of conformational change occurring in this region and it is more than likely a result of the protonation and subsequent deprotonation of residue E61.

Acknowledgements: Albert Einstein College of Medicine SURP 2010, Dr. Girvin, and Yisong Tao. Special thanks to Dr. Cahill for assistance with NMR.

A functional genomics screen for DNA elements that mediate the deposition of macroH2A

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Histone variants replace normal histones in nucleosomes in order to mark regions of chromatin for various specific purposes. MacroH2A is a large histone variant distinguished by a ~30 kDa carboxyl-terminal macro domain. Enriched on the inactive X chromosome of female mammals, macroH2A was originally suggested to be a repressive chromatin component. However, further research from our lab and others determined that macroH2A is broadly deposited over a quarter of the human genome in large chromatin domains. While macroH2A1 generally colocalizes with heterochromatin, functionally it can play either positive or negative role in transcriptional regulation in a context specific manner. While we know a lot about where macroH2A1 associates with the genome, we know very little about the cis-acting determinants that target macroH2A for deposition into chromatin. Given the size of macroH2A1-containing domains, which can span hundreds of kilobases, we hypothesize that macroH2A incorporation into chromatin is initiated by macroH2A deposition elements (MDEs), after which macroH2A incorporation propagates to nearby nucleosomes. In order to identify MDEs we used a functional genomics screen. DNA from a macroH2A1 chromatin immunoprecipitation (ChIP) of A549 lung cancer cells was used to generate a retroviral library. The library was then transduced into A549 cells and screened by macroH2A1 ChIP, followed amplification of the macroH2A1 bound library fragment by PCR. The results showed a highly significant enrichment of macroH2A1 on integrated library sequences indicating that MDEs are quite common in macroH2A1 containing chromatin. Experiments are currently underway to determine the sequence characteristics of MDEs. Overall, these experiments will lead to a greater understanding of macroH2A1 chromatin biology.

The neuroprotective properties of estradiol and its analogs in middle-aged female rats

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Previous studies have shown that estrogens are neuroprotective against cell loss after focal and global ischemia. Both pre-ischemic and post-ischemic treatment with 17β-estradiol (17β) can improve survival of pyramidal neurons in the CA1 section of the hippocampus after global ischema. Moreover, recent evidence indicates some estrogen analogs that do not bind to classical estrogen receptors (ERs) exhibit neuroprotective effects in focal (Yi et al., 2007. *Journal of Pharmacology and Experimental Therapeutics*, 324(3),1188-1195) and global (Lebesgue et al., 2010. PLOS ONE, 5,1-7) ischemia. There is a growing interest in determining the mechanisms by which estrogens attenuate ischemia-induced neuronal death and whether estrogenic compounds are effective in older patients, who are more likely to experience ischemic events. This study examined the nueroprotective properties of 17β and several of its analogs that do not bind to classical ERs when acutely administered after global ischemia to middle-aged, female rats that were ovariectomized (OVX) for 2 months before injury. OVX rats underwent sham surgery or transient global ischemia through bilateral occlusion of the vertebral arteries and the common carotid arteries. Ischemic animals were treated once, immediately upon reperfusion, with either: DMSO (vehicle), 17β-E2, ent17β-E2, ZYC3, 17α-E2, or ent-E1 (estrone). One week later, rats were killed and their brains were perfused. The brains were then sliced into 20 μm sections, mounted on slides and stained with hematoxylin and eosin. The CA1 area of the hippocampus was identified and photographed. Surviving pyramidal neurons in this section were counted. We found significant group differences in cell survival when cell counts were analyzed by ANOVA ($p<0.0001$). Ischemic animals treated with 17β-E2 and ent17β-E2 had significantly more surviving neurons in the CA1 area of the hippocampus compared to vehicle-treated ischemic rats. In contrast, ZYC3, 17α-E2, and ent-E1 did not improve CA1 pyramidal cell survival. These data demonstrate that 17β-E2 and ent17β-E2 are neuroprotective against cell death after ischemia even in older females subjected to prolonged hormone withdrawal and suggest that the neuroprotective pathway may be mediated through receptor independent mechanism.

Studies of Organic Anion Transport Protein1a1: Preparation and Expression of p3xFLAG Construct

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Organic anion transport protein1a1 (oatp1a1) is expressed on the basolateral plasma membrane of rat hepatocytes where it mediates uptake of amphiphilic molecules and xenobiotics from the blood. Oatp1a1 is a 12-transmembrane domain integral membrane protein that has a PDZ consensus site at its C-terminus and binds to PDZK1. The long-term goal of the present study is to determine whether oatp1a1 forms homooligomers. The short-term aim of this study was to prepare an expression plasmid encoding oatp1a1 in which a 3xFLAG sequence is expressed at its N-terminus.

Oatp1a1, polymerase chain reaction (PCR) amplified using primers devised to add *NotI* and *KpnI* restriction sites, was inserted into the multicloning region of the p3xFLAG expression plasmid. DH5α E.coli were transformed with this plasmid and grown on agarose plates using ampicillin as the selection marker. Minipreps of randomly selected bacterial clones were performed in which extracted DNA was digested with enzymes *NotI* and *KpnI* and checked by DNA sequencing, as indicated. Several plasmids with the correct cDNA sequence were obtained and were used to transfect HEK293T cells. Forty-eight hours after the transfection, cell lysates were prepared and subjected to Western blotting to assay for expression of 3xFLAG-oatp1a1 protein using oatp1a1 and FLAG antibodies.

P3xFLAG-oatp1a1 was prepared successfully and confirmed by DNA sequencing. Transfection of HEK293T cells with this plasmid revealed abundant expression of a protein that reacted with both FLAG and oatp1a1 antibodies on Western blot.

In summary, a plasmid expressing 3xFLAG-oatp1a1 has been prepared successfully. Co-transfection of HEK293T cells with this plasmid and a plasmid encoding oatp1a1 linked to a different marker (e.g. GFP) will permit studies to determine whether immunoprecipitation of one form of oatp1a1 will contain the other, indicating that they are bound in a complex. This plasmid should thus provide an important tool in which to conduct oatp1a1 dimerization studies in the future.

Acknowledgements: Albert Einstein College of Medicine, SERC 2010, SURP 2010, and the entire Wolkoff Lab.

Study of the Effect of NFATc1 on Heart Development

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Department of Genetics

Congenital heart disease (CHD) is the leading cause of infant morbidity in the United States. It is characterized by its defects in the hearts' structures, hence our research focuses on development mechanisms of CHD by studying how the heart develops. All the cell tissue level endocardial cells and endocardial-derived mesenchyme form the core heart structures, valves and septa. Our attention is focused on the biology of endocardial cells and their pathogenic role in CHD.

Nuclear Factor in Activated T-cells c1 (NFATc1) is the only transcription factor to date expressed by the endocardial cells during heart development. Invitrogen BrdU staining was performed to express endocardial and mesenchymal proliferating cells of wildtype and knockout 11.5 and 12.5 mice embryo. We used a genetic approach to investigate the importance of NFATc1's presence in the developing heart. Images of the knockout and wildtype embryo validate that there is a lack of development in embryo lacking NFATc1 and an earlier decrease in endocardial cells. As the embryo ages, the number of endocardial cells vs. mesenchymal proliferating cells decrease, however in my data, this process occurred earlier in the knockout embryo. Could this be part of the reason why certain development such as valve leaflets, are absent in these knockout embryo's? Future research needs to be done to determine how the lack of NFATc1 in endocardial cells affect each stage of development to further correlate its absence to CHD.

Ackowledgements: Albert Einstein College of Medicine Summer Undergraduate Research Program

Par1 Localization during Embryonic Kidney Development

by

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The glomerulus is the filtering unit of the kidney, and consists of capillary loops which are surrounded by epithelial cells called podocytes connected by slit diaphragms. During podocyte development, columnar epithelial cells of the S-shape body evolve into highly structured and polarized cells. The arborized structure of the podocyte is required to maintain the integrity of glomerular filter and is disrupted in proteinuric kidney diseases like focal glomerulosclerosis (FSGS).

The family of Partitioning defective (Par) proteins plays a role in establishing cell polarity in columnar epithelial cells and neurons by asymmetric localization of Par1 and the Par3/Par6/aPKC complex to distinct cell membrane domains. It has been shown that the Par3 complex localizes to the podocyte slit diaphragm, and that the complex is required for normal podocyte structure. We have identified expression of Par1a/b kinases in podocytes and in developing nephrons. We hypothesize that Par1a/b contributes to podocyte differentiation. The objective of our research was to examine the expression of Par1a/b during embryonic kidney development and in adult podocytes.

Immunogold labeling of Par1a in kidneys examined by electron microscopy allowed us to localize Par1a predominantly to the podocyte cell body and foot process cytoplasm. Within the foot process, the majority of Par1a localized to apical or basal aspects of the foot process, rather than at the slit diaphragm. Consistent with this, Par1a in adult glomeruli co-localized with the apical podocyte marker podocalyxin on confocal immunofluorescence. Next, embryonic rat kidney tissue was co-stained for Par1a/b and for WT-1 or Pax-2, which demarcate the metanephric mesenchyme (MM) and developing S-shape nephrons. Par1a/1b were expressed in the MM and in S-shape nephrons. Quantification of Par1a/1b expression was examined using western blotting, demonstrating increased expression in embryonic day 15 kidneys, at which time glomeruli begin to form.

Together, these data suggest that Par1a/1b may play a role in podocyte differentiation. Further studies are necessary to define Par1a/b function in the developing and mature kidney.

Acknowledgements: AECOM – SURP 2010
Roth Scholars Program,
Dr. Frederick Kaskel, MD, PhD - Program Mentor

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Acknowledgements: Albert Einstein College of Medicine–Summer Undergraduate Research Program 2010
Roth Scholars Program,
Dr. Frederick Kaskel, MD, PhD - Program Mentor

Genetic Mapping of the Cell Competition Suppressor Gene *su(comp)3L-2* in *Drosophila*

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Cell competition has been implicated in the early stages of cancer (Rhiner et al., 2009), as a potential tool in regenerative medicine (Oertel et al., 2006), and as a mechanism by which tissues optimize fitness (Moreno et al. 2002a). Cell competition can occur in gentotypic chimeras where, through intercellular interactions, one genotype ("winners") survive, while their genetically distinct neighbors ("losers") are eliminated (Baker and Li 2008). More specifically, cell competition has been observed in chimeras consisting of cells expressing varying levels of either the proto-oncogene *myc* or various ribosomal proteins. It is thought that "winner" cells produce a killing signal, initiating caspase activation and apoptosis of the "loser" cells, (Moreno et al. 2002a), in conjunction with an engulfment response by "winner" cells (Baker and Li, 2007). Mutation of the competition suppressor gene *su(comp)3L-2* has been shown to prevent the apoptosis of "loser" cells, a fundamental component of cell competition (Tyler et al., 2006). We will map the *su(comp)3L-2* mutation using Single Nucleotide Polymorphism (SNP) markers in *Drosophila* to further our understanding of the molecular mechanisms involved in cell competition.

Acknowledgements: Albert Einstein College of Medicine: Victoria Freedman, Rosa Garcia, and everyone involved in the Summer Undergraduate Research Program (SURP); Nick Baker, Abhijit Kale, John Fullard, and the entire Baker Lab

Generation of Shuttle Phasmids from Cyclops and Eureka: Mycobacteriophages Isolated from Kwa-Zulu Natal, South Africa

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Abstract.

Mycobacteriophages have played a central role in the study of tuberculosis by allowing for the genetic manipulation of mycobacterial cells. Ominously, the emergence of XDR-TB (extensively drug resistant tuberculosis) strains have brought the tuberculosis pandemic to the forefront of global health concerns, heightening the need for a rapid detection tests of tuberculosis drug susceptibility. Phage infection of mycobacterial cells is a time-efficient method for the evaluation of drug susceptibility. Using the shuttle phasmid methodology of cosmid cloning outlined in Jacobs et al. 1987[1], new shuttle phasmids were engineered from the phages Cyclops and Eureka, which were isolated from soil samples by students at the University of Kwa-Zulu Natal. Following isolation and characterization of the mycobacteriophage genomes, the cosmid pYUB328 was randomly inserted around the mycobacteriophage genomes. Transfection of this library into *M. smegmatis* allowed us to identify the non-essential regions of these previously uncharacterized phages and generate recombinant mycobacteriophage vectors. A set of different reporter genes will be efficiently introduced into these phages to generate novel reporter mycobacteriophages that infect *M. tuberculosis* cells. Drug susceptibilities of *M. tuberculosis* cells will be determined by infecting *M. tuberculosis* cells in the presence or absence of TB-drugs as cells that are killed by the drug will not permit active expression of the fluorescent protein encoded by the infecting reporter mycobacteriophage. We intend to test if these phages can improve the speed of drug susceptibility assays in Kwa-Zulu Natal. .

[1] Jacobs, W.R., et al. Introduction of foreign DNA into mycobacteria using a shuttle phasmid. Nature. 1987

AID and Gadd45a: are they Involved in Active DNA Demethylation of the 3'RR and Class Switch Recombination?

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The production of antibodies, proteins with two heavy (H) chains and two light chains, helps the body fight the large repertoire of invading pathogens. A shift in expression from the IgM antibody isotype to other classes of antibodies occurs via H chain gene DNA rearrangements in a process termed class switch recombination (CSR). CSR is regulated by a 3' regulatory region (3'RR), which acts at long distances on the H chain coding regions to promote H chain germline transcription required prior to CSR. The Birshtein lab has shown that the 3' RR undergoes progressive DNA demethylation during CSR, including an early stage of replication-independent active demethylation. A question we are addressing is whether active DNA demethylation is critical for CSR. Two proteins implicated in active DNA demethylation are activation-induced cytidine deaminase (AID), a B cell-specific trans-acting protein critical for CSR, and Gadd45a, a protein involved in genomic stress.

My first project determined whether AID was involved in demethylation of the 3'RR region. Analysis, using AID knockout mice, showed that there is no significant difference in demethylation between wild-type (WT) and AID knockout (KO) mice. My second project involved Gadd45a. Although it was previously shown that B-cells from Gadd45a KO mice had reduced active DNA demethylation in their 3' RR during switching, no defects in CSR were observed in these mice. To examine if splenic B-cells contained potential compensators that would allow CSR to occur in the presence of reduced Gadd45a expression and reduced DNA demethylation, we performed a lentivirus knockdown of Gadd45a in CH12 cell lines. Using FACS analysis, we found that there is no significant difference in switching between the control shRNA and Gadd45a knockdown.

Therefore, we conclude that (1) there is no connection between AID and active demethylation, and (2) there is no direct link between Gadd45a, demethylation and CSR.

Diversity of Plasmodium faliciparum var gene sequence among children with cerebral malaria from Malawi

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Malaria is one of the most widespread human tropical diseases, causing approximately one million deaths per year. The severity of the disease can range from mild flu-like symptoms to lethal manifestations of the disease such as cerebral malaria. The disease is an infection of erythrocytes by protozoan parasites of the genus *Plasmodium*, which are transmitted via blood-feedings of anopheline mosquitoes. Nearly all of deaths and most severe malarial infections can be attributed to the species *Plasmodium falciparum*.

In order to mediate cytoadherence to human endothelial cells the parasite expresses antigens in the cell membrane of the infected erythrocyte which are encoded by the highly polymorphic var gene family. This is a highly polymorphic gene, and often multiple transcripts are expressed in a single parasite. They encode PfEMP1 a large protein which contains multiple domains includine Duffy-Binding-Like Domains. Parasites in cerebral malaria cytoadhere to brain vasculature. Why some parasites sequester in the brain specifically and cause severe disease is unknown. Interestingly studies have shown that sequence diversity in this region of the protein has been associated with more severe outcomes.

Thus we set out to define var sequence variation of the DBLα1 domain in a cohort of highly clinically characterized children with cerebral malaria from Malawi. RNA from peripheral blood of seven children with cerebral malaria was studied. Some of these children had true cerebral malaria as represented by retinal abnormalities reflecting brain sequestration, and the rest had no evidence of brain sequestration but were infected with malaria. cDNA was generated from each sample. A 400 bp sequence was then amplified that included the DBLα1 domain sequence. Only three samples were suitable for analysis due to failure to amplify the desired sequence. These amplified products were then cloned into Topo-TA vector, transformed into Ecoli, grown overnight and DNA was isolated. From each sample we analyzed multiple clones. Within three of the samples we identified multiple different var sequences. We then compared all amino acid sequences across all three samples and found a 24 percent conservation of all the amino acids. Additionally, when compared to samples from a different study in Papua New Guinea there were several motifs that were completely conserved. Conversely we identified regions of diversity in the Malawi samples. Further studies with more subject samples could define the diversity of var DBLα1 domain in this region and indicate if there is a correlation of specific amino acid sequence and cerebral malaria and possibly mediate CNS sequestration

Acknowledgments: Johanna P. Daily, Yetunde Junaid, Claire Brown, Lakshmanan Viswanathan, Kris Subramaniam, the Diaz-Griffero Lab, the Levy Lab and SURP 2010. Additional thanks to the Sinnis Lab at NYU.

Assessment and Comparison of Detecting Methodologies of HCV Virus Genotypes 2 & 4 in at Risk Population

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To treat Hepatitis C virus (HCV) physicians administer anti-retrovirals and use the patient's HCV-RNA levels (viral load) as progress markers, making these measurements vital to patient care. Both Abbott Diagnostics and Roche Molecular Diagnostics have their own version of real time polymerase chain reaction (RT-PCR) assays that target conserved regions within the various subtypes of HCV in order to measure viral loads. Recent publications have reported that Montefiore's Roche TaqMan Amplicor assay substantially undervalues viral loads in samples of genotypes 2 and 4, possibly due to inaccurate assumptions in the conserved region for PCR. A retrospective analysis performed on the Montefiore Hospital database showed the large impact of this possibility, as Montefiore treats the large and diverse immigrant population of the Bronx. Samples from HCV genotypes 2 and 4 that have been previously tested on the current Roche assay will be processed on the Abbott RealTime HCV assay using the m2000sp instrument for extraction and the m2000rt instrument for amplification and detection. The viral loads determined by each of the assays will be compared to each other for accuracy and to themselves for reproducibility. We will use alternate (quantitative) method comparison on Data Innovation, Inc.'s program EP Evaluator® to analyze our data. The limits of agreement will be computed using Bland-Altman analysis. The results of this study will guide the decision of the Virology Center with respect to which assay to use.

This work was supported by Einstein's Summer Undergraduate Research Program (SURP) and funded by Abbott Diagnostics.

Towards local implementation of sequencing based risk assessment for the autism spectrum disorders

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The autism spectrum disorders, a class of pervasive developmental conditions afflicting 1 in 110 individuals, is typified by language, communication, cognitive, and social impairments. Although early intervention is well established to improve outcome, diagnosis relies entirely on behavior that may not manifest until two years of age or later. Molecular strategies exist and could enable earlier intervention but are cumbersome and have a low overall yield. To address these issues, we sought to develop a framework that would facilitate the selection of gene and regional content for interrogation by next generation sequencing. Two publically available databases, Decipher and OMIM, were used to identify regions and genes of potential interest. Informatics tools including the UCSC genome browser and Galaxy were then employed to integrate the output from these analyses and convert the data into genome coordinates. Results indicate that we are able to obtain genomic coordinates for genes or regions of interest and use these data to evaluate the capacity requirements for experimental interrogation by next generation sequencing. Additional work will be required to streamline this process in preparation for sequencing in patients.

Acknowledgements: Funding from SURP (S.F.K.) and AECOM (B.S.A.) made this work possible.

Understanding the Splicing Mechanism of MacroH2A1

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The histone variant macroH2A replaces the canonical histone H2A in chromatin to regulate gene expression. The H2AFY gene that codes for macroH2A1 encodes two splice variants, macroH2A1.1 and macroH2A1.2, 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 many cancer types have reduced levels of macroH2A1.1 splicing. In addition, we have recently determined that macroH2A1.1 expression 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 these mechanisms are perturbed in cancer. To begin identifying the cis-acting sequences that regulate macroH2A1 splicing, we designed a "minigene" including the two alternative exons and 300 bp of surrounding 5' and 3' intronic DNA. By expressing the minigene in human cells, we were able to reproduce accurate splicing for macroH2A1.2 but not for macroH2A1.1. Using an independent approach, we sought to identify trans-acting factors affecting macroH2A1 splicing. By correlating the level of macroH2A1 splicing in 50 tissues and cell lines with available microarray expression data, we generated a list of candidate splicing factors. We developed shRNA knockdown constructs for several candidates to determine their role in macroH2A1 splicing. Knockdown of QKI, whose expression positively correlates with macroH2A1.1 splicing, resulted in lower levels of macroH2A1.1 splicing and higher levels of macroH2A1.2 splicing. Interestingly, QKI was recently identified as a potential tumor suppressor whose expression is downregulated in colon cancer.

Funded by SURP of Albert Einstein College of Medicine and the Sidney Kimmel Cancer Foundation

The Effect of FITM2 on ER Membrane Lipid Composition

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The ability to synthesize neutral lipids and store them in the form of cytosolic lipid droplets is universal among eukaryotes. The structure, catabolism, and role in energy homeostasis of lipid droplets have been well characterized, however little is known about the mechanism of lipid droplet biogenesis.

Fat storage-Inducing Transmembrane proteins 1 and 2 (FITM1/FIT1 and FITM2/FIT2) are members of an evolutionarily conserved family of proteins that are involved in lipid droplet biogenesis. FITM2 is the anciently conserved FIT family member that is an endoplasmic reticulum (ER) resident protein composed of six-transmembrane-domains and is ubiquitously expressed in mice and humans, with the highest expression found in brown and white adipose tissue.

FITM2 has been shown to induce lipid droplet accumulation without augmenting triacylglycerol biosynthesis or lipolysis, however the mechanism by which it accomplishes this remains unknown. Here, we present our exploration of the effect of FITM2 expression on ER membrane lipid composition, specifically testing the hypothesis that expression of FITM2 enhances the concentration of triacylglycerol in ER membranes. Additionally, we take a broad-based approach and determine the effects of FITM2 expression on all classes of lipids in the ER. In order to accomplish these aims, our protocol for ER membrane isolation from cultured cells is currently being optimized. After successful isolation of ER membranes in both wild-type HepG2 cells and HepG2 cells with approximately a 90% reduction in FITM2, the ER membranes will be subjected to thin layer chromatography and lipidomics to assess the membrane lipid composition.

Acknowledgements

We thank the members of the Silver Lab and the Summer Undergraduate Research Program (SURP) at the Albert Einstein College of Medicine. We also acknowledge Dr. Erick Snapp for his contributions to this study.

Cloning and Expression of *Mycobacterium tuberculosis* CYP 121 (Mycocyclosin Synthase)

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The development of multi-drug resistant and extensively-drug resistant *Mycobacterium tuberculosis* has created an urgent need to discover treatments for this disease. The *M. tuberculosis* gene Rv2276 has been shown to be essential for *M. tuberculosis* and encode a cytochrome P450 (CYP121). CYP121 is responsible for the synthesis of a novel cyclodipeptide derivative, mycocyclosin. Cyclodipeptides are an important group of secondary metabolites and many of them have been shown to have therapeutic value against a variety of diseases. CYP121 was expressed in *E. coli* using recombinant genetics and purified. The purified protein was then used to the study the mycocyclosin biosynthetic pathway to potentially yield a novel small-molecule treatment for *M. tuberculosis*.

Acknowledgments

Subray Hegde, Brett Wolfson-Stofko, Hector Serrano, Christine Quartararo, An Vandemeulebroucke, Clarissa Melo Czekster, Hua Xu, Lee Tremblay, John Blanchard
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Autophagy in the Growth of Primary Cilia

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Mouse embryo fibroblasts (MEFs) grow primary cilia upon serum starvation for 6-48 h. At approximately the same time that primary cilia are forming during cell-starvation, the process of autophagy is occurring in the cells. Autophagy is a catabolic pathway that results in the degradation of cytosolic components inside lysosomes. The fact that autophagy and primary cilia formation are occurring in the cell at the same time suggests a possible functional relationship.

Two forms of autophagy - macroautophagy and chaperone mediated autophagy (CMA) - are induced by starvation with kinetics similar to the induction of primary cilia. To test whether ciliary growth was affected by loss of macroautophagy, we used pharmacological modulators of macroautophagy and cells from a mouse knocked out for an essential component of this autophagic pathway. All cell lines still grew primary cilia, supporting that macroautophagy is not required for cilia formation.

Interestingly, acute blockage of macroautophagy by 3-methyladenine resulted in a marked increase in the number of primary cilia even in cells grown in nutrient-rich media. Cells with chronic blockage of macroautophagy showed a similar trend, although differences with wild-type were less pronounced. Our results highlight the importance of macroautophagy in modulating the energetic cellular balance. Even if cells are growing in nutrient-rich media, when macroautophagy is blocked, cells perceive themselves as starving (maybe because of a decrease in the intracellular pool of amino acids) and will thus produce primary cilia.

Acknowledgments

I would like to thank the Roth Scholars Program and the Summer Undergraduate Research Program for this project and the members of the Cuervo lab for all their time and support in helping me conduct my research. Supported by NIH/NIA AG021904

Determination of Bucky ball pathway and binding domains via its protein interactors

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The establishment of oocyte polarity along the animal-vegetal axis is a critical process for establishing the axes of the embryo and the germline of the zebrafish embryo. The Balbiani body, an evolutionarily conserved aggregate of organelles, RNAs and proteins, is the earliest known sign of asymmetry in vertebrate oocytes. Bucky ball, a protein which localizes to the Balbiani body and has no known functional domains, is essential for the polarization of the oocyte, assembly of the Balbiani body, and formation of the first embryonic axis, as seen through the mutant phenotype. The pathway through which Bucky ball mediates this process is heretofore unknown. A Yeast-two-hybrid screen of a human ovarian tissue cDNA library was performed to identify Bucky ball interacting proteins and their binding domain on Bucky ball. Through this approach we have identified multiple, unique Bucky ball interacting proteins, many of which have been implicated in infertility and cancer, but how they contribute to these diseases is not known. Interactions between truncated versions of Bucky ball protein and its binding partners in yeast is revealing regions of the Buc protein required to mediate binding, which may also represent domains of functional significance. Validation of binding between Bucky ball and its interacting partners identified in yeast through EMSA and co-immunoprecipitation studies, and using zebrafish genetics to explore the essential functions of validated proteins will provide insight into the mechanisms establishing oocyte polarity and specification of the germline. .

Trypanosoma cruzi induced lipolysis

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Trypansoma cruzi is a protozoan parasite that causes Chagas disease which is an important cause of heart disease in endemic areas of Latin America and among immigrants from those areas in North America. It is estimated that approximately 25% of the Latin America population are at risk for this disease, which kills more then 50,000 people every year. Because there is no effective vaccine, the latest research has been aimed at studying the areas in which the parasite can persist during infection.

Infection with *T. cruzi* is accompanied by an intense inflammatory reaction. Adipose tissue is both a target and reservoir of *T. cruzi*. Adipose tissue is an endocrine organ contributing to energy homeostasis, immune response, inflammation and infection. Adipocytes and their role in the pathogenesis of infection have increasingly gained attention due to the inflammatory function of adipose tissue.

We have examined the cause for the reduction in fat mass by analyzing the lipolytic and apoptotic pathways in acute murine model using immunoblot analysis. We infected CD-1 mice with 1 x 10^4 trypmastigotes. At days 15 and 30 post infection, we sacrificed the mice and collected epididymal WAT (white adipose tissue) and BAT (brown adipose tissue) for protein analysis. We studied the signaling pathways involved in lipolysis and apoptosis using immunoblot analysis technique to measure the protein levels in infected and uninfected tissues. We found that *T. cruzi* infection is associated with an increase in lipase expression especially in WAT. We observed no significant difference in the apoptotic protein levels between infected and uninfected mice (BAT and WAT). Significant decrease in neutral triglycerides and lipids in adipose tissue during acute infection is a direct correlation with the reduction in fat mass observed in these mice. These studies suggest that the reduction in fat mass can be attributed to the lipolysis pathway in our acute murine model.

Acknowledgements
Albert Einstein College of Medicine: Summer Undergraduate Research Program 2010, Dr. Victoria Freedman, Dr. Louis M. Weiss, Dr. Streamson C. Chua, Lena Josephs, and the rest of the Tanowitz laboratory for all of their support
FASEB MARC: Lisa Silverman and Tara Snethen

Genetic Interactions between selected Exo / Endonucleases in *S. cerevisiae*

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The DNA mismatch repair system (MMR) corrects mismatches that occur during DNA replication. When a mismatch is recognized, specific nucleases excise it as an obligate step in efficient repair. *EXO1* and *RAD27* respectively encode an exonuclease and an endonuclease that function at DNA replication forks. Exo1 is involved in double stranded DNA excision, and Rad27 processes Okazaki fragments. Simultaneous deletion of both genes is lethal in budding yeast (*Saccharomyces cerevisiae*), although each single deletion is viable. The Keogh lab has also found that the comparable double deletion is lethal in fission yeast (*Schizosaccharomyces pombe*), but only after 5 – 10 generations. This suggests that another nuclease partially compensates for the function(s) of Exo1 and Rad27. The goal of my project was to identify genetic interactions between budding yeast *exo1*Δ (i.e. a deletion of *EXO1*) or *rad27*Δ and a range of candidate exo- and endo- nucleases.

High-copy plasmids containing 24 exo- and endonucleases were isolated from a tiling library created by the Prelich lab. Each plasmid was digested with restriction enzymes to confirm identity. Plasmids were then transformed into wild type (WT), *exo1*Δ, and *rad27*Δ strains and spotted onto YPD (a non-selective rich media) to determine growth-rates. A number of high-copy phenotypes were observed related to *KEM1*, *NTG2*, *RAD2*, and *RAD17*: e.g *RAD2* overexpression is synthetic with *rad27*Δ but has no effect in WT or *exo1*Δ cells; the slow-growth after *KEM1* overexpression in WT is rescued by *rad27*Δ or *exo1*Δ. Since Kem1, Ntg2, Rad2, and Rad17 are potentially involved in DNA repair, further experiments will determine whether the interactions between these factors and *exo1*Δ or *rad27*Δ are impacted by DNA damage (e.g. in the presence of methylmethanesulfonate; MMS). Future experiments will also include analyses to determine whether the overexpression of any exo- or endonucleases can rescue the lethality of a [*exo1*Δ / *rad27*Δ] double-mutant strain.

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This project would not have been possible without the help of the members of the Keogh laboratory and the 2010 Summer Undergraduate Research Fellowship funding.

Profiling Cullin Substrates Subjected to Cullin-Mediated Protein Degradation

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Calcineurin is a serine/threonine phosphatase that plays an important role in transplant surgery. Calcineurin is also believed to be involved in schizophrenia and possibly diabetes. Effectors of calcineurin, however, are underexplored. Recently, we have demonstrated that calcineurin regulates degradation of certain protein substrates. One of the key components of this protein degradation complex is scaffolding protein Cullin, which promotes the attachment of the correct F-box leading to specific substrate-binding and degradation. We have demonstrated that Cullin1 (Cul1) promotes degradation of a specific subset of calcineurin substrates. Here, I examined different members of the Cullin family (Cul2, Cul3, Cul4A, Cul4B, Cul5) in the degradation of calcineurin substrates. I found distinctive profiles of calcineurin substrates that are degraded by specific Cullins. These findings will identify novel proteins that are substrates for the calcineurin phosphatase. Special thanks to the Summer Undergraduate Research Program in the Graduate Division of Biomedical Sciences and the Department of Molecular Pharmacolgy at the Albert Einstein College of Medicine. Also, special thanks to the Albert Einstein College of Medicine mass spectrometry facility.

Proximal Control in Inducible Nitric Oxide Synthase: The effect of M186H mutation

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Nitric oxide synthase (NOS) catalyzes the formation of nitric oxide (NO) from L-arginine (L-Arg). There are three main isoforms of NOS. These include : -a) neuronal NOS (nNOS), which is a neurotransmitter for signal transduction , b) inducible NOS (iNOS), which is a cytotoxic agent for immune responses , and c) endothelial NOS (eNOS, which is a regulator for vascular function.

Like cytochrome P450, NOSs belong to the family of cysteine-coordinated heme proteins in which the proximal ligand to the heme-Fe is the sulfur atom of an intrinsic cysteine residue. The crystal structure of NOS reveals that there is a H-bond between the cysteine thiolate and the proximal Trp 188 residue. The NO generated in NOS at the end of the catalytic cycle can either diffuse out of the heme pocket to the solvent or it can bind to the heme iron thereby inhibiting the turn-over of the enzyme. The degree of the NO auto-inhibition is relatively high in nNOS (to prevent overactivity in neurons) and low in iNOS to produce large cytotoxic amounts of NO.

After analysis of the primary sequence of the three isoforms, we hypothesized that Met 186 which varies from isoform to isoform in a highly conserved helix, on the proximal side of the heme in iNOS, plays critical role in regulating the NO auto-inhibition. To confirm this hypothesis, we studied the M186H mutant of iNOS, as this residue is substituted by His in nNOS. .

Our spectroscopic and kinetic studies show that the M186H mutation significantly perturbs the chemical reactivity of the enzyme, highlighting the functional importance of this residue. It was concluded M186H behaves slightly like nNOS. However, the data from the heme transitions opposes the hypothesis by leading to significant increases in the production of NO. One new hypothesis is that the M186H protein strengthens the bond between the sulfur atom of the proximal cysteine and the heme iron. This would weaken the Fe-NO bond and increase the rate of the NO Dioxygenase reaction. Resonance Raman spectra of the M186H iNOSoxy protein will be measured to study the relative strengths for heme Fe-S bond and the heme Fe- NO bond.

Funded by Summer Undergraduate Research Program - DSSROP of Albert Einstein College of Medicine and NIGMS grant F31GM078679 (JS).

Nitrite Binding of Met (Fe^{3+}) Hemoglobin E (β26 Glu→Lys)

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Homozyotes for HbE usually have symptoms such as mild anemia, while heterozygotes HbE/β thalassemia exhibit highly morbid clinical symptoms which include severe anemia, growth and developmental retardation, transfusion requirements, and endothelial/cardiac dysfunction. In addition to the above complications, patients also exhibit oxidative stress. These manifestations have led to the question of how the structural or functional properties of HbE contribute to the clinical consequences. Recently, the Hirsch and Friedman laboratories (Roche et al., in preparation) demonstrated that deoxy (T-state) HbE has a reduced nitrite reductase activity, indicating that HbE might produce less bioavailable nitric oxide that would contribute to the clinical manifestations of oxidative stress and vascular/cardiac dysfunction. The hypothesis arose that nitrite binding to HbE is reduced in the T-state compared to HbA. We investigated nitrite binding to HbE as a function of conformational change in the T or R states of HbE. In order to manipulate the conformation of the protein, Hb was converted to the met (Fe^{3+}) form (R-state), and in the presence of reduced pH and effectors, the equilibrium was shifted to the T-state. A titration of nitrite binding to met HbE compared to HbA was monitored using absorption spectra. The results suggest (1) the T-state of HbE binds nitrite with a lower affinity compared to HbA; and (2) a possible alteration of the T-state conformation of HbE compared to HbA.

Acknowledgements: We gratefully acknowledge the SURP and DSSROP for their support (DOA). This work was supported by the American Heart Association Heritage Affiliate Grant-in-Aid 0755906T (REH).

Effect of oxidative stress on Retinal Ganglion Cells: A model for glaucoma.

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We studied the ramifications of oxygen and glucose deprivation (OGD) on retinal ganglion cells to mirror the homeostatic conditions during progressive loss of blood flow to the optic nerve, as is often associated with glaucoma, the second leading cause of blindness worldwide. There were two parts to our study. First, we wanted to determine whether OGD treatment resulted in primarily apoptosis, a form of preprogrammed cell death, or necrosis. Second, we wanted to test the hypothesis that Ca2+ entry through Ca2+-permeable AMPA receptors (AMPARs) might exacerbate the effect of OGD and increase the frequency of necrosis/apoptosis. Accordingly, we treated cultured retinal neurons overnight with an AMPA receptor antagonist, a treatment which has previously been shown to increase the percentage of Ca2+-permeable to Ca2+-impermeable AMPARs. Control and treated cells were exposed to OGD treatment for 20 minutes and then either processed immediately to measure necrosis using standard techniques, or after 24 hours to measure apoptosis. Significant apoptosis was not detected in neurons by the TUNEL assay, but there was a two-fold increase in fragmented DNA in OGD samples found outside of neurons, thereby suggesting that OGD has adverse effects of neurons. Further experiments are being conducted to ascertain if these adverse effects are caused by fast acting necrosis.

Generation of Shuttle Phasmids from Eureka and Lesedi: Mycobacteriophages Isolated from Kwa-Zulu Natal, South Africa

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Abstract.

Mycobacteriophages have played a central role in the study of tuberculosis by allowing for the genetic manipulation of mycobacterial cells. Ominously, the emergence of XDR-TB (extensively drug resistant tuberculosis) strains have brought the tuberculosis pandemic to the forefront of global health concerns, heightening the need for a rapid detection tests of tuberculosis drug susceptibility. Phage infection of mycobacterial cells is a time-efficient method for the evaluation of drug susceptibility. Using the shuttle phasmid methodology of cosmid cloning outlined in Jacobs et al. 1987, new shuttle phasmids were engineered from the phages Eureka and Lesedi, which were isolated from soil samples by students at the University of Kwa-Zulu Natal. Following isolation and characterization of the mycobacteriophage genomes, the cosmid pYUB328 was randomly inserted around the mycobacteriophage genomes. Transfection of this library into *M. smegmatis* allowed us to identify the non-essential regions of these previously uncharacterized phages and generate recombinant mycobacteriophage vectors. A set of different reporter genes will be efficiently introduced into these phages to generate novel reporter mycobacteriophages that infect *M. tuberculosis* cells. Drug susceptibilities of *M. tuberculosis* cells will be determined by infecting *M. tuberculosis* cells in the presence or absence of TB-drugs as cells that are killed by the drug will not permit active expression of the fluorescent protein encoded by the infecting reporter mycobacteriophage. We intend to test if these phages can improve the speed of drug susceptibility assays in Kwa-Zulu Natal.

Proteolysis of the Ebolavirus Glycoprotein Causes Stepwise Destabilization of the Pre-Fusion Conformation

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Ebolavirus is a filamentous enveloped virus with a negative sense ssRNA genome. Cell fusion is mediated by its lone surface glycoprotein, GP, a trimer comprised of GP1 and GP2 subunits. It is known that cathepsin proteolysis of GP is necessary for Ebolavirus infectivity. While the final triggering step involved in Ebolavirus fusion is unknown, we hypothesize that by heating the virus we can trigger fusion to determine the role of proteolysis in fusion.

Recombinant VSVΔG virus bearing wild type glycoprotein on its surface was heated in citrate phosphate buffers to non physiological temperatures ranging from 44 °C to 70°C. The rVSV pseudotypes were either uncleaved, chymotrypsin cleaved to generate an 18K intermediate, or thermolysin cleaved to generate a 17K intermediate. Half the samples were treated with the reducing agent DTT. By ELISA, the biotinylated antibody KZ52 detected a pre-fusion specific epitope at the GP1-GP2 interface.

Proteolysis caused destabilization of GP in a stepwise manner. The reducing agent DTT also caused moderate destabilization, although not to the degree of proteolysis. However, low pH, while predicted to have a destabilizing effect, did not in fact seem to have a significant effect on GP. The data suggest that proteolysis of GP and DTT treatment both destabilize the pre-fusion conformation.

Acknowledgements: Summer Undergraduate Research Program 2010 at the Albert Einstein College of Medicine, Chandran Lab: Dr. Kartik Chandran, Dr. Nirupama Mulherkar, Anthony Wong, Rohini Sandesara, Emily Miller, Onyinyechukwu Uchime

Structure and Function of Severing Proteins

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Abstract:

Microtubules can be found in the cytoskeleton of the cell. Their principle function is to provide structure and support. However, they are also dynamic structures that aid in cellular processes such as mitosis. They tend to polymerize and depolymerize producing long and short microtubules. Even though they can depolymerize on their own, there are many types of proteins that can disturb the stability of microtubules. The group of proteins currently being studied are called severing proteins. Katanin is a kind of severing protein that hydrolyzes ATP in order to break microtubules. However, the exact mechanism by which this occurs is not fully understood yet.

To address this issue, katanin- microtubule complexes were investigated through the use of electron microscopy and negative staining. Katanin was placed in three different conditions. Our goal for this experiment was to see the structure of Katanin and discover how exactly Katanin breaks a microtubule. Our results provided evidence that Katanin made more cuts in the ATP conditions, as already previously established. We found possible structures of Katanin bound to a microtubule but were not able to see how exactly Katanin cuts a microtubule. Our results showed that there is a great possibility that the structures found were the Katanin protein. Since we saw an increase in the amount of cuts in our experimental condition (Kat and ATP), this provided us with promising evidence that Katanin was responsible for the cuts. Therefore, further investigations with electron microscopy could possibly show katanin while it breaks a microtubule.

Acknowledgement: Analytical Imaging Facility and the help of Geoffe Perumal.

Human prostanoids and prostanoid glycerols are substrates for secreted lipases of *Candida parapsilosis*

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Candida parapsilosis is a normal commensal of human skin, however, infections can occur if contaminated skin is broken. Nosocomial infections frequently result from interactions between health care workers and patients. *C. parapsilosis* grows well in glucose and lipid-rich solutions and risk factors for infection include total parenteral nutrition, catheterization, prosthetics, and immunosuppression. Secreted lipases degrade host prostanoids (signaling molecules derived from arachidonic acid) which are produced in response to infection. The glycerol ester derivative of arachidonic acid, 2-arachidonoylglycerol, is the precursor to naturally produced prostanoid glycerols whose biological function is unknown. Due to their involvement with immune system regulation, enzymatic activity of lipases on prostanoids or their derivatives may divert the immune response during infection.

AutoDock software was used to simulate binding between enzymes and ligands and the most stable conformation for each class of interactions was analyzed. Interpretation of binding energies showed that lipase 1 bound prostanoid glycerols over prostanoids while lipase 2 did not consistently favor either one. Similarly, lipase 1 bound prostanoid glycerols with a lower energy than did lipase 2 while lipase 2 associated with prostanoids more strongly than lipase 1. This indicates that lipase 1 may degrade host prostanoid glycerols and, while results regarding lipase 2 are unclear, repeating the simulations with more specific parameters may yield more conclusive results. The most likely candidates could be tested *in vitro* and *in vivo* to confirm the substrates for these lipases, determine their mechanism of action, and, eventually, to devise a therapeutic intervention to minimize hospital outbreaks.

Acknowledgements:
Special thanks to Dr. András Fiser for giving me the opportunity to work in his lab and to Dr. Vilas Menon for his support and guidance this summer. Also, thank you to Rosa Garcia, Nilda Soto, Victoria Freedman, and the rest of the SURP staff for making this program possible. (Grant #8870)

Abstract

Design and Synthesis of 5,3-Substitued-1,2,4-oxadizole containing Novel Retinoids to study Retinoic acid Signaling Pathways Using Zebrafish Embryos

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In the context of our ongoing chemical biology project, studying the role of retinoic acid signaling pathways during zebra fish embryogenesis, we synthesized a small library of new 5,3-substitued-1,2,4-oxadizole containing retinoids. Retinoids (retinol [Vitamin A] and its biologically active metabolites) are essential signaling molecules that control various developmental pathways and influence the proliferation and differentiation of a variety of cell types in the adult.[1] Understanding the importance of the retinoids, we synthesized a small library of these compounds that was screened for bioactivity in living zebrafish embryos. We found that several structurally related compounds significantly affect development. Distinct phenotypes are generated depending on time of exposure, and we characterize one compound (BT10) that produces specific cardiovascular defects when added 1 day post fertilization.[2,3] This lead compound may be useful for manipulating components of retinoid signaling networks, and may be further derivatized to enhance activity and selectivity. For that purpose we undertook this project to synthesize 5,3-substituted-1,2,4-oxadizole containing novel retinoids as BT10 analogues.

Based on SAR we synthesized our BT10 analogue by replacing the conjugated alkene backbone of the BT10 molecule with a constrained phenyl ring system, we intended to avoid the metabolism of BT10 into its isomers, 9-cis-RA, and 13-cis-BT10. We also hypothesized that the methanol group could be replaced by a methoxy substituent in order to increase the efficacy of our retinoids. Further, we replaced the amide linkage with a bioisostere of the amide, the oxadizole group. These changes were intended to increase the efficiency and efficacy of our retinoids with respect to their interaction with retinoic acid receptors (RARs), and to explore new avenues of retinoic acid signaling pathways in zebra fish embryogenesis.

To synthesize these novel retinoid analogues, we first created the amidoxime reagent by reacting the desired nitrile with hydroxylamine hydrochloride in the presence of triethylamine with ethanol as a solvent. The acid reagent was synthesized by refluxing our alcohol in acetonitrile with triphenylphosphine hydrobromide, to form a Wittig salt. This Wittig salt was then reacted with Methyl-4-formylbenzoate in DMF with Sodium tert-Butoxide as a base to yield the acid component of our analogue. The amidoxime was then coupled to the acid in a one-pot reaction using EDCI as a coupling reagent. All compounds were purified by chromatographic separation and accessed by ^{1}H and ^{13}C NMR spectrum analysis, as well as HRMS analysis. The resulting library of BT10 analogues will be screened for bioactivity in zebra fish embryos to determine their viability as transcriptional modulators.

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Irving Rosario Jr., Keith Z. Hazleton, Quan Du, Keith Clinch, Douglas R. Crump, Peter C. Tyler, and Vern L. Schramm Phosphoribosyl transferase: Assay Development and Inhibition Study Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461

Phosphoribosyl transferases (PRTases) are enzymes involved in purine, pyrimidine, and amino acid synthesis. These processes are needed in order to sustain life, as such long term deficiencies can cause serious disease while short term disruption is a chemotherapeutic target for many diseases. Hypoxanthine-guanine-xanthine phosphoribosyl transferase (HGXPRT) is an enzyme crucial in the purine salvage pathway. Complete deficiency in this enzyme causes Lesch-Nyhan syndrome, a disease characterized by hyperuricemia and neurological problems such as mental retardation. Less severe enzymatic deficiency leads to gout. *Plasmodium falciparum* is a parasitic protozoan and a major agent of malaria. Similar to many parasitic protozoans, *Plasmodium falciparum* has no *de novo* synthesis pathway for purines and relies on the salvage pathway to gain the necessary purines for DNA and RNA synthesis. HGXPRT is a key enzyme in this pathway. Transition state analogues have been developed in order to inhibit HGXPRT to purine salvage by *Plasmodium falciparum*, preventing parasitic growth. Various inhibitors have been previously created and were tested using *Pf*HGXPRT, *Pv*HGXPRT, and *Hs*HGPRT. K_I values were determined for all the enzymes using five different inhibitors. Results show that these inhibitors bind with an approximately 500-fold tighter affinity for *PfHGXPRT* as compared to human HGPRT. Our lab is also currently developing a luciferase assay to measure pyrophosphate production by PRTases. Buffer for the assay has been purified by HPLC and charcoal columns, but there is still contaminating pyrophosphate present.

Optimizing the Detection of •OH Footprinted DNA

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Knowledge of the structure of biopolymers allows for the expansion of our understanding of its function. Footprinting assays are relatively inexpensive methods, both in terms of time and cost, of determining this structure compared to high-resolution alternatives. The small size and high reactivity of the hydroxyl radical makes it a good footprinting probe to determine the solvent accessible surface area of DNA by cleaving the backbone with single nucleotide resolution. Hydroxyl radicals can be produced by the Fenton reaction, where H_2O_2 and Fe(II) form •OH. It was recently reported that pyrite (FeS_2) can act as a source of •OH radicals.

The overall goal of this project is to develop a high-throughput method for hydroxyl radical footprinting. One part of this endeavor is the optimization of the fragment detection. We use the fluorophore Cy5 which is attached to the DNA either before or after cleavage. This is detected by a capillary electrophoresis (CE) system. DNA cleavage was achieved by i) the standard Fenton chemistry, or ii) pyrite chemistry using a microfluidic device. The target DNA was doped with either calf thymus DNA (ctDNA) or tRNA prior to the cleavage reaction in order to prevent complete degradation of the DNA.

Our results suggest that the amount of target DNA injected into the CE system is reduced as the concentration of ctDNA present increases. Since labeling of DNA fragments was achieved by Cy5-primer technology, the temperature for annealing was varied until an optimal temperature of 50°C was found. It retains the full length DNA at a two-fold increase over annealing at 55°C, and at a four-fold increase over annealing at 60°C. For both labeling methods, pyrite induced cleavage profiles with single-nucleotide resolution correspond to results derived from standard Fenton reaction generated •OH. Using directly Cy5 labeled dsDNA, solvent accessibility data can be conveniently generated without additional primer extension technology.

Funding for this research was provided by the Albert Einstein College of Medicine Summer Undergraduate Research Program, Roth Program (YS) and NSF Grant # 0852796 (MB, JS).

Generating Functional 5-HT_{3A}-$GABA_A$ Receptor Chimeras Containing the $GABA_A$ M3M4 Loop To Study the Subunit Dependence of $GABA_A$ Receptor Trafficking

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γ -aminobutyric acid type A receptors ($GABA_A$Rs) are heteropentameric neurotransmitter-gated ion channels involved in fast synaptic inhibition in the CNS. The cytoplasmic loop between the M3 and M4 transmembrane domains is thought to be involved in receptor trafficking and localization in neurons. To study the role in receptor localization of the $GABA_A$R M3M4 loops from rat β2 and γ2 subunits we generated chimeras inserting the β2 or γ2 M3M4 loop into the mouse homomeric serotonin receptor, 5-HT_{3A}. The initial chimeras were nonfunctional. We hypothesized that this might be due to the choice of splice sites. To alter the splice sites we tried to insert 3, 5, or 7 alanines at the sites. Inserting three or five alanines preceding the M3M4 loop in the β2 chimera was insufficient to produce functional channels. Inserting three alanines following the $GABA_A$ γ2-M3M4 loop in the chimera elicited currents from the injected oocytes, with rapid desensitization. The 5-HT EC_{50} value was 3.8 μM in the mutant, compared to the 1.0 μM in wild-type channels. The C-terminal region of the $GABA_A$ γ2-M3M4 loop is rich in positively charged amino acids. The alanine insertion increases the separation of these charged residues from the cytoplasmic end of M4 thereby reducing possible electrostatic or steric interactions between the cationic residues and the membrane or cytoplasmic end of the transmembrane segments. The functional chimeric channels can now be imaged in transfected neurons to assess the role of the individual $GABA_A$ subunit's M3M4 loop in receptor localization.

Acknowledgements: Albert Einstein College of Medicine Summer Undergraduate Research Program, 2010; IJ Frame, Rishi Parikh, Moez Bali, Julia Goldberg, Julia Rappoport, Asif Rahman, Avish Arora.

Correlation Between Serum and Plasma Antibodies to Mycobacterial Antigens

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Background: Many cases of active tuberculosis (TB) are challenging to diagnose, especially those that occur in HIV+ individuals. In these cases, serodiagnosis in the form of detection of antibodies (Abs) to immunodominant antigens of *Mycobacterium tuberculosis* (MTB) could be an ideal adjunct test to diagnose TB earlier. Traditionally serum, which does not contain fibrinogen and clotting factors (unlike plasma), is used to test for Ab responses to MTB, though it would be beneficial to be able to utilize serum or plasma samples interchangeably. However, to our knowledge no studies have compared the Ab responses to mycobacterial or other antigens detected in serum versus plasma.

Methods: To determine whether levels of serum and plasma Abs correlate, simultaneously obtained serum and plasma samples from TB and non-TB patients, were tested by ELISA for both IgG and IgA Abs to two immunodominant proteins or a polysaccharide antigen of MTB. Results were correlated using the Spearman rank test.

Results: A very strong and highly statistically significant correlation (average $r = 0.924$; $p<0.0001$ for all data sets) was found between serum and plasma Ab responses for both IgG and IgA and to all 3 mycobacterial antigens tested. In subgroup analysis for TB+/TB- and HIV+/HIV- samples, the correlation remained strong and statistically significant.

Conclusion: Serum and plasma samples can be used interchangeably to test for Ab responses to mycobacterial antigens, even in the same assay.

This work was supported by the Roth fellowship to MS and the NIH grant AI-067665 to JMA.

Title: Development of a Listeria-poliovirus vaccine to treat cancer at young and old age

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Cancer is a disease of the elderly. However, elderly react less efficient to vaccines than young adults, due to lack of naïve T cells (react for the first time to new antigens). One way to address this problem is to develop a *Listeria monocytogenes* (LM)-based vaccine expressing recall antigens. Most individuals have been exposed to recall antigens such as tetanus toxoid (TT), measles virus, poliovirus (PV) etc, during childhood through vaccination, and developed memory cytotoxic T lymphocytes (CTL) to these antigens. Recently, our lab discovered that the Listeria bacteria selectively infect tumor cells (and macrophages). We hypothesize that immunizing elderly cancer patients with Listeria-recall antigen will reactivate memory (CTL) against recall antigens (generated during childhood) without the need of naive T cells at old age, and that these memory CTL will kill the infected tumor cells now highly expressing recall antigens. To test this hypothesis, we will develop a Listeria vaccine expressing PV antigens (LM-PV). First, a DNA-fragment carrying immunodominant epitopes of the PV has been amplified by polymerase chain reaction (PCR) from pTM7, cloned into pCR2.1, and analyzed by restriction digestion and DNA sequencing. Subsequently, the PV fragment will be subcloned into a listerial plasmid pGG34 (pGG34-PV), and finally transfected into a highly attenuated LM (XFL-7). Once the LM-PV has been generated, we will test the LM-PV vaccine in young and old mice with breast cancer, and expect that PV-specific memory CTL (generated at young age) reactivated by vaccination with LM-PV will reduce tumor growth at both ages.

Acknowledgements
This work was supported by SURP and a NIA/NCI-funded grant (1 R01 AG023096). We thank Swathi Krishnan for helping Lukman with initial experiments.

Single Nucleotide Polymorphism Associations in TBX1 in Individuals with 22q11.2 Deletion Syndrome

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The 22q11.2 deletion syndrome (22q11.2DS) is characterized by a 1.5-3 mega base deletion on chromosome 22. Children with 22q11.2DS exhibit a wide range of clinical features. TBX1, a member of the Tbox family of transcription factors, maps within the region of 22q11 deleted, and has been proposed as a candidate gene for some of the features in this condition. Polymorphisms in the non-deleted TBX1, which may affect the function of the remaining TBX1 gene in individuals with the 22q11.2DS, may be a key to understanding the phenotypic variability observed in individuals with a shared deletion.

Thirty-one single nucleotide polymorphisms (SNPs), both in and around the TBX1 gene region, were selected for genotyping with 2000 DNA 22q11.2DS samples. An initial group of 650 patients with the 22q11.2DS were genotyped for these SNPs to identify genetic variants that influence their phenotype. To accomplish this, the samples were organized, diluted to a proper concentration, amplified for the specific SNP regions of interest, and genotyped. Genotype information was obtained using the Sequenom iPLEX Gold assay.

To date, 650 DNA samples were successfully genotyped. The association analysis is ongoing and not yet completed. Once all the 2000 DNA samples have been genotyped, a full association study can be performed.

This work explores the complex relationship between genotype and phenotype and can potentially provide a model to explain clinical variability associated with this and other microdeletion syndromes.

Funding provided by the Roth Fellowship 2010, Summer Undergraduate Research Program 2010 of the Albert Einstein College of Medicine and the NIH.

Does A Rapid POC PT/INR Test Improve Patient Outcome? A Retrospective Database Analysis of the Montefiore Experience

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Patients on anticoagulants such as Coumadin must carefully monitor their Prothrombin Time (PT), a measurement of the time it takes for blood to clot. Coumadin Management is a delicate balance between preventing clotting and bleeding. Prior to the advent of POC Roche CoaguCheck blood testing at Montefiore Medical Center (MMC) samples were sent to the Core Lab to be tested. Patients had the option of waiting an average of 4 hours for their results and then seeing their physician, or leaving and having their results and dose changes reported via phone call. Under the current POC testing system finger-stick whole blood samples are analyzed by the Roche CoaguCheck a small, portable device that obtains results in under 5 minutes. MMC standards accept Roche CoaguCheck INR values up to 3.5. Due to internal studies that showed discrepancies between the Roche CoaguCheck and the gold standard Core Lab, all values >3.5 are sent to Core Lab for confirmation. Roche CoaguCheck POC testing has provided a convenient INR testing method for the physician, decreased turn-around-time between testing, and resulted in a greater degree of face-to-face management between patient and physician. This study will evaluate and verify that POC PT/INR leads to an increased duration in the therapeutic range (2.0-3.0) as compared to historical controls.

This work was supported by Einstein's Summer Undergraduate Research Program (SURP).

The histone demethylase Lid's function in *Drosophila melanogaster* somatic mutation accumulation

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The trimethyl histone 3 lysine 4 (H3K4me3) demethylase Little imaginal discs (Lid) in *Drosophila melanogaster* acts as a proposed co-activator for the proto-oncogene dMyc. The binding of Lid is required for dMyc-induced expression of growth regulatory genes. Deregulation of dMyc causes cancer through increased cellular growth, faster cellular divisions and an increase in somatic mutation frequency. Transgenic strains of *D. melanogaster* overexpressing dMyc have a significantly higher level of insertions, deletions and genome rearrangements. To observe whether the increase in mutation frequency is a consequence of Myc being in the presence of Lid, DNA of third instar larvae from transgenic *D. melanogaster* strains with a UAS/Gal4 system driving the overexpression of Lid was collected. Antibody staining of larval fat bodies and wing imaginal discs with α-Lid and western blot analysis confirmed the overexpression of Lid protein. A rescue assay was performed with the isolated DNA digested with *Hin*dIII. DNA was eluted and circularized with ligase T4. Precipitated DNA was transformed into *Escherichia coli* and plated on P-gal and on X-gal. Mutation frequency was determined as the ratio of colonies growing on the P-gal plates versus the number of colonies found on the X-gal plates. There was no significant difference in mutation frequency between wild type flies and Lid overexpressing mutant flies. This suggests that Myc is responsible for increasing the accumulation of mutations and that the Lid protein alone does not contribute to causing an increase in somatic mutation frequency.

Acknowledgements

This project was supported by the 2010 Albert Einstein College of Medicine Summer Undergraduate Research Program to CS. Authors would like to thank Chrissy Greer for technical assistance and Maaike Westerhof from the Vijg lab for help with rescue assay performance.

The Effective Use of MRI and microPET Co-registration in Mice and Rat Studies

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MRI and microPET images have been used separately to study the growth of tumors in mice and rats. However, they can be superimposed on each other to more accurately compare the images and draw conclusions about the tumors and the size of organs based on where they overlap.

Rats and mice were imaged with the MRI and then injected with [^{18}F] before being put in the microPET machine. The computer programs of MATLAB 7.1, ASIPro, and Amira 4.0 were then used to label and co-register the two different images. It was possible to co-register organs such as the brain, eyes, and kidneys. Imaging these animals proved that it is possible to use MRI and microPET co-registration to accurately compare organ and tumor size.

This method can be used to further identify possible tumor growth not only in rodents, but in other animals as well.

Acknowledgements: SURP 2010, Institute for Animal Studies, Dr. Eugene Fine, Dr. Craig Branch, Dr. Elaine Lin

Optimization of the Copper-Catalyzed Click Reaction for *in vitro* Protein Labeling

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Click chemistry offers a selective and efficient method for bioconjugation. The copper-catalyzed azide-alkyne cycloaddition (CuAAC), referred to as the "cream of the crop" of click reactions by nobel laureate K. Barry Sharpless, has become the standard for bioorthogonal conjugation. However, free copper (I), the reaction catalyst, is toxic to cells and is a strong reducing agent, and thus, copper-coordinating ligands are required for stabilizing the copper (I) oxidation state and for sequestering copper (I)-associated toxicity. TBTA, one of the original ligands used in CuAAC, has poor water solubility and slow kinetics in promoting the reaction. This project examines the effectiveness of alternative copper-coordinating ligands in labeling glycoproteins in cell lysate, the efficacy of the new ligands for reducing amino acid oxidation for proteomic analysis, and the ability of these ligands in preventing cellular apoptosis in the presence of free copper (I). Western blotting experiments showed that the new ligands studied are suitable for catalyzing azide-alkyne cyclizations in cell lysates, although the optimal ratio of ligand to copper varies. Determination of protected-histidine modification by LC-MS analysis revealed that the ligands significantly reduce the amount of amino acid oxidation in the presence of copper (I). Examination of cell death by flow cytometry showed that the ligands are effective in reducing apoptosis of Jurkat cells during the click reaction. Thus, the ligands studied in this project appear to be effective for use with the CuAAC in bioconjugation.

Funded by Albert Einstein College of Medicine SURP and the National Institutes of Health

GPotential Hepatitis C Restriction Through Trim5α proteins

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Hepatitis C is an infectious disease transmitted through blood-to-blood contact and affects an estimated 300 million people worldwide. Cyclophilin A (CypA) has been identified as a cofactor of the virus and is necessary for replication. Cyclosporine A, an inhibitor of CypA, has shown to have anti-HCV activity and reduces the virus's ability to replicate.

Trim5α proteins in primates and TrimCyp in the owl monkey block infection of HIV-1 and other retroviruses by promoting premature decapsidation and accelerated uncoating of the HIV capsid. TrimCyp is a derivative of other Trim5 α proteins, the difference being a domain of Trim5 α is replaced by a CypA domain. TrimCyp's ability to restrict HIV-1 and other retroviruses is thought to occur through the binding of the CypA domain to the capsid. This idea is reinforced by the decrease of restriction by TrimCyp when cyclosporine is present. Due to HCV's dependence on CypA for replication, we hypothesize that the TrimCyp protein will restrict infection in a similar mechanism that occurs with certain retroviruses.

A protocol was developed to ensure that the human hepatocyte cell lines (Huh-7.5) were successfully and stably transduced with one of the thirteen Trim 5 proteins that were chosen before being exposed to Hepatitis C Virus (HCV). After successfully establishing the cell lines, the next step is to infect them with HCV and determine if any of the Trim5 proteins are able to restrict infection.

The discovery of the Trim5α proteins ability to restrict HIV-1 has led to extensive research of this protein family. Since monkeys can be infected with Simian Immunodeficiency Virus (SIV) without getting the monkey equivalent of AIDS, the discovery of a protein that restricts infection post-entry was very significant. If this family of proteins were able to restrict HCV as well, the findings would open new door for HCV research.

Objective Measure of Subjective Rhythm as Evidenced by EEG

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When tones of the same intensity are played in a regular pattern, the brain will automatically amplify some of the tones to strong beats. This study's purpose was to collect cerebral evidence to show that rhythm could be subjectively accented and induced by a preceding auditory context. Patterns of binary and ternary rhythms have been induced in previous experiments, but those experiments were only behavioral and induced the rhythm through a movement such as bouncing. This study used adult human participants who listened to a pattern of tones that induced either a binary or ternary rhythm, and then had a pattern of tones that was the same in both rhythms, but could be accented differently depending on the induced rhythm. Softer sounds would be played during the last or second-to-last tone in the ambiguous part. The participants reported when they heard the softer sounds, while their EEG was recorded on their scalps. Mismatch negativity (MMN) and P3 were measured to determine if the ambiguous sequence was induced to sound rhythmically different. MMN was found to be larger in amplitude when the beat was strong. P3 was nearly equal in magnitude through all the deviants. This questions the relationship between perceptual salience of a tone due to its temporal position and its loudness due to physical sound level.

This work was supported by SURP and an NIH grant.

Word count: 227

Cited3 Promotes Differentiation of Oxidative Muscle Fibers

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In vertebrates, muscle development occurs through the sequential segmentation of mesodermal tissue into repetitive structures called somites. Muscle formation and differentiation in the somites is controlled by various signaling pathways. Recent spatiotemporal microarray studies have identified novel, uncharacterized transcription factors which might be involved in muscle differentiation and/or fiber-type specification. Cited3 is one such novel gene encoding a transcriptional cofactor. We hypothesize that Cited3, which is expressed in the oxidative fiber precursors of zebrafish embryos, promotes the development of oxidative fibers.

In this study, Cited3 expression was knocked down in zebrafish embryos by injecting morpholino oligonucleotides that block the translation of Cited3. The phenotypic effects on morphology and muscle fiber formation of these morphants were investigated at 30hpf. *In situ* hybridizations for various muscle-specific genes were also performed on wild type and Cited3 morphant embryos to determine whether such genes are under Cited3 regulation.

Reduction in Cited3 expression results in morphological abnormalities such as an edema in the yolk sac and a tail curvature. Slow fiber-specific immunostaining shows a reduction in slow fiber myogenesis in the trunk region of Cited3 morphants. The *in situ* results demonstrate that znf238 and stnnc are unaffected by the loss of Cited3 whereas prox1 and α-actinin expression are reduced in Cited3 morphants. Based on these findings, we conclude that Cited3 may be involved in myofibrillogenesis in oxidative, slow-twitch muscle fibers.

Acknowledgments: This research was supported by the 2010 Roth Fellowship and the Summer Undergraduate Research Program at Albert Einstein College of Medicine.

Hysteresis of Voltage Gating in Gap Junctions Channels: Evidence for a Novel Deep-Closed State for the Slow Gate of Apposed Hemichannels

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Gap junctions (GJ) channels create a direct pathway for metabolic and electrical cell-cell signaling and are composed of two apposed hemichannels (aHCs), each of which is composed of six connexin (Cx) subunits. It is established that aHCs exhibit two types of gating: fast and slow. Dysfunction of these gating mechanisms has been linked to cardiac arrhythmias and a host of other Cx-related congenital diseases.

Recently, we have observed hysteresis in the junctional conductance-junctional voltage (g_j-V_j) dependence, which contradicts the widely accepted view that GJ channels gate between two states, open and closed. In addition, a Stochastic Four State Model (S4SM) built on this two-state principle does not predict the hysteresis observed. However, it can be reproduced if the S4SM is built to have two closed states: the initial-closed state (*i-c*) and the deep-closed state (*d-c*), wherein the slow gate is permitted to move from the open state to the *i-c* state and from the *i-c* state to the *d-c* state, but the transition from the open state to the *d-c* state is prohibited. Acidification is postulated to promote the transition of the slow gate to the *d-c* state. Thus, acidification is expected to increase hysteresis, since the increased probability of the transition to the *d-c* state results in a delayed recovery of the channel to the open state. Furthermore, alkalization should cause an opposite effect by the same mechanism.

Cells expressing wild-type Cx40 and Cx43 were used to examine the effects of pH on g_j-V_j hysteresis with the dual whole-cell patch clamp method. We found that alkalization by exposing cells to 3 and 5 mM NH_4Cl decreased hysteresis by, on average, ~23% and ~42%, respectively, and that acidification by exposing cells to a solution saturated with 4% CO_2 (pH_i=~6.1) increased hysteresis by ~115%. Thus, these data support the existence of a *d-c* state, the probability of the transition to which from the *i-c* state is modulated by pH. Future investigation would include the clarification of the Cx-isoform dependent magnitude of hysteresis-pH relationship for other Cx isoforms.

Acknowledgements: **Albert Einstein College of Medicine:** Summer Undergraduate Research Program 2010, Feliksas Bukauskas and Nicolas Palacios-Prado for scientific discussion…. and Angele Bukauskiene for technical support

Endogenous inhibitor of apoptosis ARC promotes TNF-alpha induced necrosis.

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Cell death is an essential process in development, homeostasis and pathogenesis. Apoptosis and necrosis are two major forms of cell death. Apoptosis is known to be regulated through a complex signal transduction network. Necrosis was previously thought to be an uncontrolled form of cell death. However, recent works suggest that necrosis can be regulated. TNF-alpha is a pleitropic molecule implicated in cell survival, apoptosis and necrosis. Fas-Associated protein with Death Domain (FADD) and caspase-8 are two important players in the TNF-alpha mediated apoptotic cell death. Apoptosis repressor with caspase recruitment domain (ARC) is a unique repressor of apoptosis and was previously shown to interact with FADD and caspase-8. We suggest that by interacting with FADD and caspase 8, ARC inhibits apoptosis and promotes necrotic cell death through the TNF-alpha pathway. To test this hypothesis, immunoprecipitation experiments were performed using hemagglutinin (HA)-tagged ARC in cells under necrotic treatment conditions. Although we have not been able to show this interaction conclusively, cell death assays indicate that ARC promotes TNF-alpha induced necrosis. Propidium iodide (PI) staining was used to score for cellular membrane disruption under different treatments. Under the condition where cells are known to die through apoptosis, the presence of ARC reduced the extent of cell death significantly as expected. However, under TNF-alpha treatment alone, which has been shown to induce necrosis in L929 cells, the presence of ARC greatly increased the amount of cell death as compared to the control cells. Thus, this result suggests that ARC can inhibit apoptosis while inducing necrosis.

Acknowledgements: SURP 2010, Albert Einstein College of Medicine, Gloria Kung, Richard N Kitsis and the Kitsis lab. This work was funded by SURP and NIH grant T32AG23475.