

**Welcome Students, Faculty, and Visitors
to the 18th Annual Student Research
Symposium
of the
Graduate School of Biomedical Sciences
at NJMS**

Sponsored by the Graduate Student Association at GSBS-Newark

The Graduate School of Biomedical Sciences proudly welcomes you to our event. Each year the symposium provides an invaluable platform for the exchange of ideas and information in our scientific community. In addition to the contributions from our student scientists, we have the pleasure of welcoming **Dr. Brian O'Rourke**, professor of the Division of Cardiology, Department of Medicine at Johns Hopkins University as our keynote speaker this afternoon. Please join us for his talk entitled **“Mitochondrial Dysfunction in Cardiovascular Disease”**.

We would like to thank the GSBS Office of Admissions and Student affairs, our Program Directors, Faculty Judges and our Keynote Speaker for helping and participating in our Symposium!

The GSA

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Symposium Program

10:00 am – 12:00 pm Poster Viewing (Grand Foyer)

10:am – 11:00am Faculty evaluate odd number-posters

11:am – 12:00pm Faculty evaluate even number-posters

12:00 pm – 12:45 pm Luncheon Social (Grand Foyer)

12:45 pm – 3:15 pm Student Oral Presentations (MSB-556)

“Photoreceptor growth is responsive to Topographic cues”

Frank Kung

Biomedical Engineering Graduate Program

“Maternal immune stimulation during pregnancy facilitates prenatal immuno-developmental changes leading to a pro-inflammatory phenotype in offspring”

Milli Mandal

Department of Molecular Pathology and Laboratory Medicine

“Phenotypic Manifestations of Id1 and Id3 Ablation in the Adult Heart”

Corey Chang

Department of Cell Biology and Molecular Medicine

“Telomere Dysfunction Induced Cellular Senescence in Human Breast Cancer Precursor Lesions”

Jessica Kaplunov

Department of Microbiology and Molecular Genetics

“D1 dopamine receptor post-transcriptional regulation in cocaine addiction: investigating the role of microRNAs”

Krishna Tobon

Department of Pharmacology and Physiology

“Spheroids: an in vitro model to study quatiopotential, PDGF $\alpha\alpha$ responsive, progenitors of the rat subventricular zone”

Lisamarie Moore

Department of Neurology and Neuroscience

“A Key Role for CARM 1 Arginine Specific Methylation in Vitamin D Receptor Mediated Transcription”

Leila Mady

Department of Biochemistry and Molecular Biology

“Human Salivary Cystatin SA exhibits antibacterial effect against the periodontopathogen, *Aggregatibacter actinomycetemcomitans*”

Ganeshnarayan Krishnaraj

Department of Oral Biology

“Investigating the Impact of Helminth Co-Infection on CD8 T Cell Immunity”

Andrew Marple

Department of Medicine

3:15 pm – 3:30pm Coffee and Refreshments (Grand Foyer)

3:30pm – 4:30pm Keynote Address (MSB-556)

“Mitochondrial dysfunction in cardiovascular Disease”

Brian O’Rourke, M.D., Ph.D.

Division of Cardiology, Department of Medicine, Johns Hopkins University

5:00pm – 7:00pm Dinner and Awards reception (Grand Foyer)

Biomedical Engineering

Bogumila Swietek

Brooke Odle

Frank Kung

Hamid Bagce

Ishnoor Sidhu

Jony Sheynin

Priyanka Shah

Sara Fazelinik

Soha Saleh

Bogumila Swietek

A Shock Wave Delivery System to Induce a Traumatic Brain Injury in an Animal Model

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

There is currently no standard animal paradigm to illustrate the neuropathological changes in the brain following an exposure to an explosion such as an IED blast. This lack of study can be partly attributed to the safety issues associated with laboratory equipment used to generate shock waves. The intent of our shock wave delivery device is to induce a blast related traumatic brain injury (bTBI) in small animals in typical laboratory settings. Unlike past animal studies that employed the use of bulky equipment and use of chemical compounds which required special facilities, our shock wave delivery system is based on air-gun technology which only requires compressed air and is tabletop ready.

Mentor: Bryan Pfister

Brooke Odle

OpenSim Shoulder Model for Manual Wheelchair Users with Tetraplegia

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

Manual wheelchair users with spinal cord injury are at risk for developing shoulder injuries. The risk may be greater for individuals with tetraplegia, as they only have partial innervation of shoulder, scapular, and thoracohumeral muscles. Biomechanics and computer graphics-based models have been used to understand the relationship between wheelchair propulsion and upper limb pain. Biomechanical studies have investigated shoulder kinematics, kinetics, and electromyography (EMG). Computer graphics-based models have been utilized to input biomechanical data to generate simulations of wheelchair propulsion. The significance of a computer graphics-based shoulder model is that it serves as an active visual representation of the movement, as opposed to passive plots of shoulder kinematics and muscle activity, from which movement patterns had to be interpreted. There is a need for a computer graphics-based model of the shoulder for wheelchair users with tetraplegia. Our aim was to build upon previous studies to develop an OpenSim shoulder model for wheelchair propulsion in individuals with tetraplegia. OpenSim is open-source software that incorporates kinematic, kinetic, and EMG data as inputs to generate a dynamic simulation. The novelty of our model is that it is the first model to include fine wire EMG and an upper limb joint coordinate system in accordance with the International Society of Biomechanics standards. Our model could potentially impact the clinical diagnosis, cause and treatment of shoulder injury for manual wheelchair users with tetraplegia.

Mentor: Trevor Dyson-Hudson

Frank Kung

Photoreceptor growth is responsive to Topographic cues

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

In retinal degenerative diseases the death of photoreceptors (PRs) causes irreparable vision loss. Current attempts to replace the photoreceptor layer include transplantation of both embryonic and adult PRs. However, these techniques have had limited success mainly due to the lack of synaptic connectivity between the transplanted photoreceptors and the host tissue. One potential reason for this might be the absence of appropriate guidance cues from the host to help PRs integrate into the host tissue. Just as retinal ganglion cells are sensitive to gradients of guidance cues to lead their axons to their correct synaptic partners within the tectum, neuritic sprouting in PRs may be sensitive to positionally specific cues. Therefore, our hypothesis is that topographic cues affect photoreceptor sprouting.

In order to examine this hypothesis, eyes were obtained from adult salamanders and the cornea removed. Dextrantetramethyl rhodamine and fluorescein in salamander media were applied for 24 hours to the right and left eyecup respectively. The eyecups were then split into nasal and temporal halves, the retinas removed, and enzymatically digested with papain. Retina halves were then gently triturated and cells from the different halves were mixed and cultured on patterned Sal-1 substrates created using PDMS based microfluidic channels 30 μm wide for 1 week. Cultures were then fixed, and photoreceptors analyzed for neuritic contacts and varicosity formation.

Nasal cones were found to form contacts with other nasal cells 15.21% more often than with temporal cells while Temporal cones were found to form contacts with other temporal cells 11.35% more often than with nasal cells. Amongst rod cells, no significant differences were seen. Our data suggest differences in targeting preferences between cells depending on their nasal or temporal origin as well as between cone and rod cells. However, to rigorously test growth preferences amongst rod cells, a more controlled growth environment is necessary. We have devised a microfluidic device to control the intercellular distance between cells on our patterned substrate. By restricting targeting choices and precisely controlling intercellular distance, we expect to decrease variability in our results and to increase confidence in our data.

Mentor: Ellen Townes-Anderson

Hamid Bagce

Effects of visuomotor discordance in virtual reality on online performance and motor cortex excitability in patients with Stroke.

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

We have previously demonstrated that visuomotor discordance alters online motor performance and enhances primary motor cortex (M1) excitability in healthy individuals. Here, we hypothesized that stroke patients would exhibit similar online changes. Six chronic stroke patients were instructed to flex (P1-5) or extend (P6) the metacarpophalangeal (MCP) joint of their paretic index finger to a kinesthetically-defined 45° target angle. A flat-screen monitor was placed in the horizontal plane above the patients' hands, preventing direct line-of-sight of hand movement. Real-time visual feedback of movement was displayed by VR-rendered hand models, actuated by kinematic datagloves worn by the subjects. Visuomotor discordance was provided by applying a visual scaling factor [either G0.25, G1.75, or G1.00 (veridical control)], to the virtual finger in real-time as subjects performed the task. Twenty-two trials of each condition were randomly interspersed in one single block. On every trial, single-pulse Transcranial Magnetic Stimulation was applied to the lesioned M1 when the subjects' MCP reached 40°. Resulting motor evoked potentials (MEPs) were recorded from the first dorsal interosseous muscle of the paretic hand. Outcome measures included MEP amplitude, as well as final angular displacement, instantaneous angular velocity upon TMS stimulation, and the mean normalized jerk index to quantify movement smoothness. The joint angle and angular velocity ($F=0.541$, $p=0.598$) remained invariant across the three visual feedback conditions at the time of TMS stimulation. However, the final displacement angle was significantly different among the visual feedback conditions ($F=5.911$, $p=0.020$) with movements being 20.8% greater in the G0.25 condition ($p=0.024$) and 36.2% Jerkier ($p=0.011$), relative to veridical. In line with this, MEP amplitude significantly differed across conditions ($F=6.075$, $p=0.019$), with the G0.25 condition showing MEPs to be bolstered by 19.6% ($p=0.017$) over the veridical condition. Smaller increases were also noted in the G1.75 condition though this change was not significant ($p=0.097$). Our data demonstrate that visuomotor discordance can augment online motor performance and increase M1 excitability in patients with chronic stroke. This suggests that implementing visuomotor discordance in our novel VR environment may be an innovative methodology to alter performance and improve functional outcome and neural reorganization after stroke.

Mentor: Eugene Tunik

Ishnoor Sidhu

A MICROFLUIDIC CULTURE FOR TWO POPULATIONS OF DORSAL ROOT GANGLIA FOR DIFFERENTIAL STAINING

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

Microfluidics is poised to have an impact on life science research. With excellent properties such as miniaturization, integration, and automation, microfluidics creates new opportunities for the spatial and temporal control of cell growth and environmental stimuli in vitro. We have developed a novel microfluidic device constructed from poly(dimethylsiloxane) using soft lithography to label two adjacent populations of dorsal root ganglia (DRGs) differently. We used a passive pumping mechanism that exploits the surface energy stored in a liquid droplet to induce liquids to flow in microchannels. The principle governing the liquid flow is based on the difference in size between two droplets placed at the entrance and exit of a microfluidic channel—due to the difference in surface tension, the liquid will flow (maybe unintuitively) from the smallest to the largest droplet. This passive pumping technique was used to stain differently two DRGs cultured in connected channels. This study can find application in studying and manipulating locally the environment of aggregates of cells—such as embryonic bodies and cellular spheroids—found in the same culture environment.

Mentor: Raquel Perez-Castillejos

Jony Sheynin

Computerized Task for Studying Human Avoidance

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

Although avoidance behavior is studied extensively in animals and is a common feature of anxiety disorders, including post-traumatic stress disorder (PTSD), there are few studies investigating avoidance learning in humans. In this project, we use a computerized task to study conditioned avoidance learning in human subjects, based on a previously published task by Molet et al. (2006). In this task, subjects learn to destroy enemy spaceships with the goal of increasing their score. Some cues are associated with the launching of a bomb that will hit the participant's spaceship and produce a significant reduction in the score (warning cues), while other cues predict the bomb will not appear ("safety" cues). In order to receive a high score, subjects learn to avoid the aversive bomb hit by hiding the spaceship during the warning cue presentation, before the bomb's appearance. The task also includes an extinction phase, where the warning cue is not followed by a bomb. Preliminary results replicate those of Molet et al. (2006) and show that healthy young adults are able to discriminate between the warning cue and the "safety" cue, acquire an avoidant behavior in response to the warning cue and exhibit a temporal discrimination with higher avoidance response towards the end of the warning signal. Currently, we are collecting data from older healthy individuals, as well as from individuals who self-report PTSD symptoms. Preliminary results suggest that different populations adapt different degrees of avoidance behavior in response to the same cues. In addition, we are studying correlations between patterns of avoidance behavior and self-assessed personality measures, such as the State/Trait Anxiety Inventory (STAI), Adult and Retrospective Measures of Behavioral Inhibition (AMBI and RMBI, respectively), Tridimensional Personality Questionnaire (TPQ), Beck Depression Inventory (BDI) and the PTSD checklist (PCL). Seeking these correlations will help us understand which measures are associated with avoidance behavior and hence, might represent risk factors for developing anxiety disorders. In future work, we plan to expand the current task to further test additional aspects of avoidance behavior including contextual learning, escape-avoidance behavior pattern and the influence of safety signals on avoidance learning. Such design will parallel the animal avoidance work (e.g., Beck et al. 2010, 2011) and hence, promote translational research in the field of vulnerability factors in anxiety.

This work was supported by NSF/NIH Collaborative Research in Computational Neuroscience (CRCNS) Program and by NIAAA (R01 AA018737).

Mentor: Catherine E. Myers

Priyanka Shah

Integrity of Medial Visuomotor Pathway Predicts Better Recovery in Neglect Patients Receiving Prism Adaptation Treatment

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

Objective: To determine whether lesion location predicts functional recovery in right-hemisphere damaged (RHD) patients with neglect following prism adaptation therapy (PAT).

Background: PAT can produce substantial improvements in spatial neglect. Our previous studies suggested that PAT improved spatial bias in the motor-exploratory domain, which may be subserved by frontal cortico-subcortical systems. Therefore, the current study examined whether frontal lesion involvement determined treatment response to PAT in post-stroke patients with spatial neglect, using motor-exploratory improvement in the Catherine Bergego Scale (CBS-ME) as the outcome measure.

Design/Methods: Lesions of RHD patients (N=21) receiving PAT were manually mapped from clinical images (CT or MRI) using MRIcro. Based on the lesion maps, we extracted information of frontal vs. no-frontal lesion involvement and conducted a multilevel modeling analysis (MLM).

Results: After controlling for age and lesion size, MLM revealed that patients with frontal lesions demonstrated better improvement trajectory (n=13) than patients without frontal involvement (n=8). Therefore, we pursued a more detailed analysis on lesion-symptom relationship to elucidate which frontal-subcortical systems strongly support PAT response. The PAT-responsive group had greater integrity of 1) subcortical vs. cortical and 2) medial vs. lateral structures.

Conclusions/Relevance: Our results showed that the medial visuomotor pathway critical for visually-guided movement may support PAT response. PAT may manipulate the network for visual sensorimotor adaptation, subserved by the spared thalamus and basal ganglia, which in turn may improve motor-exploratory behaviors related to spatial neglect. It is still unclear from our preliminary data whether dorsolateral prefrontal regions may account for other performance variables.

Mentor: Anna M. Barrett

Sara Fazelinik

Involvement of Medial and Lateral Entorhinal Cortex in Delay Eyeblink Conditioning Paradigm of Latent Inhibition in Rats

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

The entorhinal cortex (EC) is a primary source of input to the hippocampus. In a previous study, our lab found a double dissociation between hippocampal and entorhinal functions: EC lesions eliminated latent inhibition (LI) of classical eyeblink conditioning, but did not interfere with spatial learning in water maze. In contrast, hippocampal lesions hindered spatial learning but did not affect LI. LI refers to the retardation in learning of the association between a conditioned stimulus (CS) and an unconditioned stimulus (US) following the repeated non-reinforced presentations of the CS alone. The current study focuses on the involvement of the EC in LI by examining the contribution of its two major divisions, medial entorhinal cortex (MEC) and lateral entorhinal cortex (LEC). Recent studies found that neurons in LEC exhibit very little spatial selectivity, whereas MEC neurons are spatially tuned and are involved in path integration, suggesting that the two divisions process qualitatively different types of information.

In the present study, male Sprague Dawley rats received either an MEC or an LEC ibotenic acid lesion or sham surgery. Following recovery, a delay eyeblink conditioning paradigm was used to assess latent inhibition. During the pre-exposure phase, rats received either 30 trials of CS alone (82dB, 500ms white noise, 25 - 35s ITI) or were placed in the conditioning box for the same duration without any CS presentation. Pre-exposure was immediately followed by paired CS-US conditioning (100 trials per session for four consecutive sessions) with co-terminating CS and US (10V, 10ms stimulation) and an average inter-trial interval of 30 sec. Our preliminary data shows that damage of the LEC impairs latent inhibition of eyeblink conditioning. In contrast, MEC lesion rats continued to show latent inhibition similar to what is seen in shams. These preliminary results suggest that the two regions of the entorhinal cortex may encode and process different information characteristics in eyeblink conditioning paradigm of latent inhibition.

Mentor: Kevin Pang

Soha Saleh

Mirror feedback in virtual reality elicits ipsilesional motor cortex activation in chronic stroke patients

Biomedical Engineering, University of Medicine and Dentistry of New Jersey, GSBS, Newark

The aim of this study was to test if mirror-visual feedback, presented through a virtual reality environment, could be used to bolster the activity of the lesioned motor cortex in chronic stroke patients. Ten stroke subjects participated in an event-related fMRI study in which they performed a simple finger movement using the non-paretic hand. During scanning, an fMRI-compatible virtual reality-motion capture interface was used to record their hand movement and actuate in real-time virtual hand models, which were presented in first person perspective as virtual feedback. Motion of the virtual hands was manipulated by either actuating the hand model corresponding to the moving (unaffected) hand (veridical feedback) or the opposite (mirrored) virtual hand. Two additional types of feedback, in which the virtual hands were replaced with moving non-anthropomorphic shapes, served as control conditions. Offline analysis of glove data showed that subjects maintained consistent movement kinematics across conditions. In each of the ten stroke subjects, mirrored feedback led to significant activation of the ipsilesional sensorimotor cortex, despite the affected hand remaining motionless during the task. Moreover, an additional control experiment and conjunction analysis confirmed that the part of the motor cortex that was activated by mirrored feedback overlapped with the area of motor cortex involved in movement production of the affected hand. Our data suggest that mirrored visual feedback may be a feasible modality that can be used to recruit select brain regions in stroke patients as a means of facilitating neural reorganization and recovery.

Mentor: Sergei Adamovich

Department of Biochemistry and Molecular Biology

Ashley Cornett *

Erica Pimenta

Fuhua Xu *

Ganapathy Sriram

Justyna Korczeniewska *

Jyoti Joshi *

Khanhquynh Nguyen

Leila Mady

Lisong Yang

Wen-I Tsou

Wenting Luo

Zhe Ji

Ashley Cornett

RHAPA: A New Method to Assess and Quantify Alternatively Polyadenylated mRNA Transcripts

Department of Biochemistry and Molecular Biology, Biomedical Sciences (Interdisciplinary), University of Medicine and Dentistry of New Jersey, GSBS, Newark

3' end formation of eukaryotic mRNAs is an essential process that influences mRNA stability, turnover, and translation. Polyadenylation is the process by which mRNAs are cleaved at a specific site and subsequently acquire a poly(A) tail. Alternative polyadenylation occurs when multiple poly(A) sites are present in the primary mRNA transcript, in either the 3'UTR or other sites within the mRNA. Alternative polyadenylation results in multiple transcripts arise of varying lengths. Current analysis of alternative polyadenylation is limited to two techniques: northern blotting and RT-PCR. Northern blotting analysis exploits usage of a radiolabeled probe hybridizing to a given complementary sequence within the RNA molecule of interest; however, it provides only a relative analysis of alternatively polyadenylated transcript expression. RT-PCR provides a more quantitative method of analyzing RNA expression; however, this quantitation is still a relative measure. We demonstrate here a new method, called RHAPA, that employs conventional RT-PCR coupled with oligonucleotide hybridization and RNase H treatment to directly measure alternatively polyadenylated transcripts. This method gives an absolute expression of each transcript and provides a way to examine poly(A) site selection in different cell types and under different conditions. Ultimately, it can be used to further examine post-transcriptional control of gene expression.

Mentor: Carol Lutz

Erica Pimenta

Role of IRF5 in the mammary tumor microenvironment

Department of Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, GSBS, Newark

Breast cancer is a heterogeneous disease whose progression from atypical ductal hyperplasia (ADH) to ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) is regulated by the aberrant expression of multiple mediators produced by the mammary tumor itself and the adjacent reactive stroma (1). These signals promote tumor cell proliferation, survival, vascularization, invasion and ultimately metastasis to secondary organs. The ability of the tumor to create a state of local immune suppression allows tumor cells to evade clearance by the immune system (2). New signaling pathways of the interleukin (IL) family, interferons (IFN) and interferon regulatory factors (IRF) have recently been found within the tumor microenvironment and in metastatic sites. Some of these cytokines stimulate while others inhibit breast cancer proliferation and/or invasion (2). The role of these cytokines and their mediators in disease progression, as markers of disease stage, and as novel treatment strategies requires further attention. We recently demonstrated that expression of the transcription factor IRF5 is uniquely down-regulated in human archival tissue specimens from patients with different stages of DCIS and IDC, which is distinct from family member IRF1; loss of IRF5 preceded that of IRF1 and correlated with increased invasiveness (3).

IRF5 is a critical mediator of the host immune response to pathogens and cellular response to DNA damage. It has recently been shown to play a major role in the polarization of macrophages, with high levels of IRF5 expression yielding M1 (pro-inflammatory) macrophages and low levels yielding M2 (anti-inflammatory) macrophages (4). Interestingly, we found that although IRF5 expression was lacking in the majority of tumors from patients with DCIS and IDC, the infiltrating immune cells, such as macrophages, displayed enhanced IRF5 expression (3). Given that the interaction between tumor cells and macrophages has been shown to be important, i.e. tumor associated macrophages (TAMs), IRF5 may contribute to tumor immune evasion in this microenvironment. The goal of this project is to further examine the differences in IRF5 expression between mammary tumor cells, the surrounding stroma, and infiltrating immune cells in order to elucidate its role in the complex tumor microenvironment. Determining the pathways and mechanisms by which IRF5 influences the tumor microenvironment will likely lead to the discovery of novel molecular targets for the treatment of breast cancer.

Mentor: Barnes

Fuhua Xu

Differential Requirement of SWI/SNF Chromatin Remodeling Complexes in Osteoblast Commitment and Differentiation

Biomedical Sciences (Interdisciplinary), University of Medicine and Dentistry of New Jersey, GSBS, Newark

The commitment of the osteoblastic lineage, and further differentiation to osteoblasts and ultimately to mature osteocytes, is tightly regulated. Successive changes in gene expression patterns are controlled by specific transcription factors such as Runx2 and Osterix, acting in concert with histone modifiers and ATPase-dependent SWI/SNF chromatin remodeling complexes. BAF and PBAF, distinct subclasses of mammalian SWI/SNF complexes, share most subunits but are distinguished by unique subunits: ARID1A/B belong specifically to BAF and ARID2 is exclusive to PBAF. The ATPase BRG1 can power either complex. Elucidating the mechanism of how BAF and PBAF differentially regulate genes required to osteoblast commitment and differentiation will be a significant step toward understanding bone development.

We have recently identified several gene expression patterns dependent on BRG1. Here we have considered whether these patterns segregate with BAF vs PBAF. Our results show induction of the osteogenic marker, alkaline phosphatase, is impaired in ARID1A or ARID1B depleted pre-osteoblasts in a milder version of the BRG1 depletion phenotype. ARID2 stable knockdown cells likewise fail to induce alkaline phosphatase and osteocalcin activity, implying non-redundant requirements for both BAF and PBAF in activation of osteogenic gene expression. Pre-osteoblasts express high levels of FGFR2, which is essential for maintaining osteoblast commitment status. Depletion of either BRG1 or ARID2 causes dramatic down-regulation of FGFR2 expression, suggesting PBAF is required for osteoblastic lineage commitment as well as for differentiation. Another phenotype of BRG1 depletion is inappropriate expression of the mature osteocyte markers *Dmp1* and *Phex* in pre-osteoblasts, indicating that a BRG1 complex is required to repress premature expression of genes specific to the terminally differentiated osteocyte phenotype. Depletion of ARID2 does not cause premature expression of *DMP1* or *Phex*, indicating that repression of osteocyte-specific genes in pre-osteoblasts is a BAF-specific function for which PBAF is not required. Collectively, these data identify some overlapping and some distinct functions for BAF and PBAF. To date, PBAF has segregated only with activation functions, while BAF participates in both activation and repression.

Mentor: ELIZABETH MORAN

Ganapathy Sriram

Non-canonical Signaling Pathway for Crk Mediated by Tyrosine Phosphorylation of the C-terminal SH3 Domain

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The SH2 and SH3 domain-containing adaptor proteins Crk, Grb2 and Nck participate in the organized assembly of protein complexes downstream of tyrosine kinases and/or their substrates. Crk uniquely functions as an oncogene and in recent years, Crk over-expression has been shown to positively correlate with the aggressive phenotypes of several human cancer types. Also, Crk knockdown attenuates the invasion and migration of patient derived cancer cell lines. Hence, there is an urgency to understand the mechanisms by which Crk promotes transformation in the hope that new information can be exploited to develop therapeutics. The canonical signaling paradigm involves the Crk SH2 binding to specific phosphotyrosine motifs at focal adhesions or the plasma membrane and the SH3N binding to polyproline motifs of proteins. Our results suggest that Crk has an unconventional role in signal transduction through phosphorylation at Y251 in the RT-loop of the SH3C domain, thereby recruiting SH2/PTB containing proteins to initiate non-canonical signaling pathways. Phosphorylated Y251 on Crk promotes Abl kinase transactivation by binding to and displacing the Abl SH2. By generating phospho-specific antibodies specific for pY251, we identified that Y251 is rapidly phosphorylated, concomittant with Abl activation, upon induction of the EGFR signaling axis by EGF in MDA-MB-468 human breast cancer cells. Mutation of the Abl binding site on Crk did not affect Y251 phosphorylation downstream of EGFR suggesting that it is Abl independent and may mediate Abl activation in this axis. Further, SH2 domain profiling reveals several potential binding partners of pY251. Identification of the functional significance of pY251 on the Crk SH3C is likely to provide unique insight into how Crk promotes the aggressive phenotypes of cancer cells.

Mentor: Raymond B. Birge

Justyna Korczeniewska

Interaction of the COP9 signalosome with IRF5 controls protein stability.

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Interferon Regulatory Factors (IRFs) are a family of transcription factors that play significant roles in innate immunity and tumor suppression. One of the newer members to be identified is IRF5. IRF5 is known to be involved in the host immune response to extracellular pathogens and plays a critical role in DNA damage-induced apoptosis and death receptor signaling pathway. Activation of IRF5 in the cytoplasm is induced by the phosphorylation of serine and threonine residues causing IRF5 homo- or hetero-dimerization. This results in translocation of IRF5 to the nucleus and subsequent targeting of promoter sequences to specific genes causing initiation of transcription. The exact mechanism by which IRF5 translocates to the nucleus and the function of nuclear/cytoplasmic IRF5 is not fully understood. Likewise, very little is known about IRF5 interacting partners. Thus, a search was performed to identify novel IRF5 binding partners using a proteomics approach.

FLAG-tagged IRF5 was overexpressed in 293T cells and immunoprecipitated. This was followed by 1D or 2D gel electrophoresis and subsequent identification of binding partners by mass spectroscopy. It was found that the subunits of the Constitutive Photomorphogenesis (COP) 9 signalosome (CSN) protein complex are able to interact with IRF5. This interaction was further confirmed by co-immunoprecipitation and western blotting in exogenous and endogenous systems. The IRF5 interaction with COP9 subunits has proven to be specific. For example, CSN4 interacts with IRF5 and not other IRFs. Fine-mapping of the IRF5/CSN interaction by immunoprecipitation with IRF internal deletion mutants demonstrates that COP9 subunits (except CSN3) interact with carboxyl terminus of IRF5 (aa 477-488). Additionally, we demonstrate that COP9 has another distinct interaction site on IRF5. COP9 CSN3 directly interacts with amino terminus of IRF5 (aa 63-72) and serves as a platform for all other CSNs. This suggests that the amino terminus of IRF5 interacts first with CSN3, then with the rest of the subunits.

COP9, a highly conserved protein complex, is found in all higher eukaryotes. It contains eight core subunits, CSN1-8, in order of decreasing molecular weight. The CSN is a multifunctional protein complex associated with many different biological activities. One well known function of CSN is regulation of protein stability through the ubiquitin-proteasome system. We have shown that the COP9 signalosome controls IRF5 protein stability. This was determined by overexpression of full length GFP-IRF5 and mutant (GFP477-488) which were then tested for protein stability by treatment with cycloheximide (CXH) and subsequent flow cytometry/western blot analyses. This data suggests that the interaction of the COP9 signalosome with IRF5 controls protein stability, however, our understanding of the exact mechanism of action is still limited. Future studies will be performed to find out if ubiquitination followed by proteosomal degradation is involved in IRF5 stability. Taken together, the loss of the COP9 /IRF5 interaction decreases the stability of IRF5 indicating that COP9 constitutively controls stability of cytoplasmic IRF5 protein.

Mentor: Betsy Barnes

Jyoti Joshi

Inhibition of human multiple myeloma proliferation by naltrindole

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The purpose of this study was to test and characterize the antiproliferative activity of naltrindole (Nti), a selective delta opioid receptor antagonist, toward human multiple myeloma (MM) cells. Nti has been shown to inhibit the allogeneic mixed lymphocyte reaction in vitro, and to block graft rejection in vivo, similarly to cyclosporine A. Based on its immunosuppressive properties we decided to test the effects of Nti on proliferation of MM cells. MM is an invasive and incurable plasma cell neoplasm responsible for 10% of all hematological malignancies. We have screened a variety of opioid compounds for activity against MM and we found that Nti inhibited the proliferation of several human MM cell lines (U266, RPMI 8226, ARP1) with an EC₅₀ of approximately 20 μ M, whereas a variety of other human cells lines were substantially less sensitive. Co-culture of MM cells with human bone marrow stromal cells did not affect the antiproliferative activity of Nti. A 10-fold molar excess of naltrexone, a non-selective opioid antagonist, did not block the Nti-induced inhibition of U266 cell proliferation. [3H]-Nti exhibits saturable, low affinity binding to intact MM cells and the pharmacological properties of the Nti binding site differ significantly from the properties of the delta opioid receptor, leading us to hypothesize that Nti inhibits proliferation of MM cells through a non-opioid receptor-dependent mechanism. RT-PCR assays confirmed the lack of delta, kappa and mu receptor mRNA in U266 and RPMI 8226 MM cells. The identity of the naltrindole binding site is currently under investigation. Nti does not induce apoptosis in MM cells, based on FACS analysis and caspase cleavage assays. While investigating the mechanism of action of Nti, we have observed that it increases intracellular calcium levels in MM cells, and the calcium appears to be released from the endoplasmic reticulum, based on inhibition of the response following thapsigargin treatment. This effect is specific to Nti as other opioids such as naltrexone and morphine do not affect the levels of calcium in MM cells, nor do they block the activity of Nti. Based on the observed in vitro anti-proliferative activity of Nti toward MM cell lines, an in vivo study was conducted. Nti injected IP daily at 30mg/kg significantly decreased tumor volumes in a murine

SCID/human RPMI 8226 xenograft model over a 39-day period compared with saline injected controls. Curcumin, a constituent of turmeric extracted from the rhizomes of the plant *Curcuma longa*) has been shown to induce apoptosis in MM cells. We have also observed that curcumin inhibits the proliferation of MM cells. Although the chemical structures of Nti and curcumin differ substantially, curcumin inhibits Nti binding with an EC₅₀ of 58 μ M in MM cells. It appears that Nti and curcumin warrant further study as potential therapeutic agents for the treatment of human MM. Supported by grants from NIDA, the NJ Commission on Cancer Research, and the Foundation of UMDNJ.

Mentor: Richard Howells

KhanhQuynh Nguyen

TAM Receptor Tyrosine Kinases Expressed on Breast Tumor Cells Regulate Efferocytosis

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In the tumor microenvironment, at least three major types of cells can phagocytose apoptotic tumor cells, a term now called efferocytosis. These cells include myeloid-derived macrophages and dendritic cells (DCs), as well as neighboring viable tumor cells. To date while most of the work in this field has focused on the role of macrophages and DCs in tumor phagocytosis, there is a great need to understand the role of tumor cells as phagocytes since it has been shown that tumor cells overexpress phagocytic receptors on their surface. Homologous receptor tyrosine kinases, Tyro-3, Axl, and Mer (collectively known as TAMs), are three receptors expressed on phagocytes that are actively involved in phagocytosis and inflammatory cytokine suppression. Additionally, these receptors are often associated with highly malignant and invasive cancers and poor patient survival. Our preliminary data indicated that Mer expression was elevated in breast cancer tissue. To examine the effects of TAM expression on phagocytosis and immune suppression, we first ectopically expressed Mer in HEK 293 cells. We found that Mer, when transfected into HEK cells, stimulated the net phagocytosis of apoptotic Jurkat cells. When exploring the relationship between TAM expression on breast cancer cells (BCCs) and their phagocytic capacity, we found that the more invasive BCCs MDA-MB231 (which overexpressed Mer and Axl) had higher phagocytic capacity over non-transformed cells. Moreover, the highly metastatic mammary tumor cell line 4T1 also expressed higher level of Axl and Mer as compared to non-malignant mammary cells HC11 and showed extremely high phagocytic activity. Also, the phagocytic capacity of MDA-MB 231 and 4T1 was compatible to that of professional phagocytes RAW264.7. In addition, efferocytosis by cancer cells 4T1 was able to suppress LPS-induced TNF- α secretion from RAW264.7. Taken together, our data suggests that breast cancer cells are active phagocytes and TAMs may be involved in the phagocytosis of apoptotic tumor cells. In the future, we plan to knockdown TAMs in BCCs and examine if this can suppress tumor tolerance.

Mentor: Raymond Birge

Leila Mady

A Key Role for CARM 1 Arginine Specific Methylation in Vitamin D Receptor Mediated Transcription

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Hormone dependent transcriptional regulation involves chromatin remodeling by coactivator proteins. Although roles of acetylation and phosphorylation in chromatin remodeling have been widely studied, recent findings have also indicated an important role for methylation. Little is known however about the exact role of methyltransferases and their regulation by different physiological signaling pathways. Using renal proximal or distal convoluted tubule cells or VDR transfected COS-7 cells and the rat 24(OH)ase promoter we found that CARM1 (a methyltransferase that methylates histone 3 at arginine 17) cooperates with the p160 coactivator GRIP1 to enhance 1,25(OH)₂D₃ induced transcription. Transfection of GRIP1 enhanced 1,25(OH)₂D₃ induced transcription 2 fold and co-expression of GRIP + CARM1 resulted in a 4-9 fold enhancement. All activity was dependent on 1,25(OH)₂D₃. A CARM1 mutant lacking methyltransferase activity failed to enhance 24(OH)ase activity in cooperation with GRIP1. In addition, when the GRIP1 mutant (Δ AD2) which lacks the binding site for CARM1 was used, cooperative activation was also not observed. This result supports a role for GRIP1 as a primary coactivator or bridge to recruit the secondary coactivator, CARM1. Thus, the coactivator function of CARM1 requires methyltransferase activity and coexpression of GRIP1. When PRMT2A, another arginine methyltransferase, was substituted for CARM1, cooperative transactivation with GRIP1 was not observed, suggesting a preferential role of CARM1 in VDR transactivation. Moreover, through ChIP-seq analysis we identified genome wide co-occurrence of VDR binding and histone 3 arginine 17 methylation in kidney cells in response to 1,25(OH)₂D₃, further supporting a fundamental role of CARM1 in VDR mediated transcription. In addition to 24(OH)ase, CARM1 co-activator activity was also observed for 1,25(OH)₂D₃ induction of TRPV6 and osteopontin. However, we found that cAMP or calcitonin transcriptional induction of 1 α (OH)ase (involved in the synthesis of 1,25(OH)₂D₃) is inhibited by CARM1 in proximal convoluted tubule cells. Thus CARM1 can act as an activator or repressor depending on the context of transcription factors at a specific promoter. Our findings indicate for the first time that CARM1 methylation may have a broad yet fundamental role in modulating target genes associated with the vitamin D endocrine system and thus in the control of the biological function of vitamin D.

Mentor: Sylvia Christakos

LISONG YANG

Interferon Regulatory Factor 5 (IRF5) controls monocyte recruitment in a model of chronic inflammation

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The transcription factor interferon regulatory factor 5 (IRF5) has been identified as a human systemic lupus erythematosus (SLE) susceptibility gene by genome-wide association studies. Exactly how IRF5 contributes to SLE development is not known; however, recent data indicate a critical role for IRF5 in the production of pathogenic autoantibodies in mouse models of lupus. In order to address more mechanistically how mice lacking *Irf5* are protected from lupus, we used the well-established model of pristane-induced murine lupus that displays many of the key immunologic and clinical features of human SLE. Data from our lab indicates that *Irf5*^{-/-} mice are deficient in their recruitment of monocytes to the peritoneal cavity (PC) in response to pristane, which is believed to be one of the initial key events leading to pathogenesis of lupus in this model. The observed defect in monocyte recruitment in *Irf5*^{-/-} mice could be due to an intrinsic defect in *Irf5*^{-/-} monocytes and/or an extrinsic defect(s) in the milieu of cytokines/chemokines expressed in the PC. Data from transwell assay using peritoneal lavage from pristane-injected *Irf5*^{+/+} and *Irf5*^{-/-} mice as the chemoattractant supported an intrinsic defect in *Irf5*^{-/-} monocytes, although an extrinsic defect may also exist. Examination of chemokine receptor expression from bone marrow monocytes of *Irf5*^{+/+} and *Irf5*^{-/-} mice by qPCR indicated that *Irf5*^{-/-} monocytes are deficient in *cxc3*, *cxc4* and *ccr5* expression. Flow analysis of protein receptor expression confirmed a defect in CCR5 expression in *Irf5*^{-/-} monocytes and showed further differences in CXCR3, CXCR4 and CCR5 in response to pristane. Preliminary data on the functional consequences of altered receptor expression suggests that the decreased migration of *Irf5*^{-/-} monocytes to peritoneal lavage may not be due to defects in response to CCL2 and CXCL12 ligands, although these data need to be further confirmed in pristane-induced monocytes. In summary, our current data support an intrinsic role for IRF5 in monocyte recruitment to the PC of pristane-injected mice; this defect is likely to contribute to the mechanism(s) of protection from lupus onset in *Irf5*^{-/-} mice.

Mentor: Betsy Barnes

Wen-I Tsou

THE APOPTOTIC CELL RECOGNITION RECEPTORS, TYRO3, AXL AND MER SHOW DISTINCT PATTERNS OF LIGAND-INDUCIBLE RECEPTOR ACTIVATION.

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Proper recognition and removal of apoptotic cells by professional phagocytes such as macrophages and dendritic cells lead to the active suppression of inflammatory responses and the induction of tolerance, and is associated with increased production of anti-inflammatory cytokines such as TGF- β , IL-10, and down-regulation of pro-inflammatory cytokines such as TNF- α , IL-12, and IL-6. Tyro3, Axl and Mer (TAM) receptor tyrosine kinases recognize apoptotic cell through their ligands, Protein S and Growth-Arrest-Specific Gene 6 (GAS6), which in turn interact with phosphatidylserine (PS) exposed on the surface of apoptotic cells. Although three TAM receptors have very similar extracellular and intracellular domains, it is currently unknown whether each of these receptors has a unique function in the recognition of apoptotic cells. Specifically, very little is known about the specificity of interaction between TAM receptors and their ligands, Protein S and GAS6, in the context of apoptotic cells. In addition, downstream signaling cascades triggered through TAM receptors were only partially characterized which makes the comparison of TAM-mediated signaling events a challenging task.

To study ligand-receptor interaction, we generated a series of reporter cell lines expressing various chimeric receptors composed of the extracellular domain of each TAM receptor, and the trans-membrane and intracellular domains of IFN γ R1 chain of the IFN γ receptor complex. This strategy allowed us to compare the interaction of each ligand and apoptotic cells with Tyro3, Axl and Mer chimeric receptors by detecting JAK-STAT (Janus Kinase – Signal Transducers and Activators of Transcription) signaling events. We found that each TAM receptor demonstrates a unique pattern of interaction with GAS6. Moreover, the presence of apoptotic cells has a different effect on the activation of each receptor by the ligand. These studies suggest that despite their similarity, Tyro3, Axl and Mer are likely to perform distinct functions in the recognition of apoptotic cells and the induction of tolerance.

Mentor: Sergei Kotenko

Wenting Luo

Transcriptional activity regulates alternative cleavage and polyadenylation.

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Genes containing multiple pre-mRNA cleavage and polyadenylation sites, or polyA sites, express mRNA isoforms with variable 3' untranslated regions (UTRs). By systematic analysis of human and mouse transcriptomes, we found that short 3'UTR isoforms are relatively more abundant when genes are highly expressed whereas long 3'UTR isoforms are relatively more abundant when genes are lowly expressed. Reporter assays indicated that polyA site choice can be modulated by transcriptional activity through the gene promoter. Using global and reporter-based nuclear run-on assays, we found that RNA polymerase II is more likely to pause at the polyA site of highly expressed genes than that of lowly expressed ones. Taken together, our results indicate that polyA site usage is generally coupled to transcriptional activity, leading to regulation of alternative polyadenylation by transcription.

Mentor: Bin Tian

Zhe Ji

Evolution and regulation of alternative polyA sites in mammalian genomes revealed by deep sequencing

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Alternative cleavage and polyadenylation (APA) leads to mRNA isoforms with different protein coding regions and/or 3' untranslated regions (3'UTRs), impacting diversity of proteome and mRNA metabolism. APA is highly regulated under various biological conditions, such as differentiation, proliferation, and development and when cells respond to environmental signals. Accurate mapping of the complete repertoire of cleavage and polyadenylation sites, or polyA sites, in the genome is important for studying APA. We have developed a high-throughput method, named 3' Region Extraction and Analysis by Deep Sequencing (3'READS), which not only addresses outstanding issues leading to false identification of polyA site, but also quantitatively measures polyA site usage. Using 3'READS, we found that APA is pervasive in the mouse genome, affecting more than 70% of mRNA genes and more than 50% of genes encoding long intergenic non-coding RNAs (lincRNAs). While APA contributes mainly to the 3'UTR diversity of mRNAs, APA of lincRNAs is frequently coupled with alternative splicing. Interestingly, evolution of polyA sites located in 3'-most exons is constrained by GC content and gene-to-gene distance. For genes that are closely spaced, the polyA site usage pattern is consistent with general avoidance of overlapping transcription. In addition, creation of polyA sites located in introns correlates with fast gene evolution, contributing to species-specific isoform production. Moreover, dynamic APA is observed in cell models of myogenesis and adipogenesis. Consistent with downregulated expression of 3' end processing factors, strong polyA sites tend to be more used when cells differentiate resulting in a general lengthening of 3'UTR and more skipping of intronic polyA sites. Knockdown of CstF64, a key 3' end processing factor, leads to global change of APA but does not fully recapitulate the regulation in cell differentiation, indicating multiple mechanisms are in play for APA regulation.

Mentor: Bin Tian

Department of Biochemistry at Montclair State University

Agnieszka Chojnowski

Agnieszka Chojnowski

The role of *Brugia malayi* stress-activated protein kinases, Bm-MPK1 and Bm-JNK1, in parasite anti-oxidative stress responses

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Pathogenic filarial nematodes affect the lives of millions of individuals worldwide along with an estimated one billion individuals at risk of infection in endemic areas. In spite of some successes in treating filarial disease with ivermectin, albendazole and diethylcarbamazine, new drugs are needed. We have been studying protective responses of the filarial parasite, *Brugia malayi* (*B. malayi*) against reactive oxygen species (ROS). ROS are produced by innate leukocytes such as, macrophages, eosinophils, neutrophils, etc. during infection. Our focus has been on two evolutionary conserved stress-activated protein kinases in *B. malayi*, Bm-MPK1 (an ortholog of human p38 mitogen-activated protein kinase) and Bm-JNK1 (an ortholog of human c-Jun N-terminal kinases). We used a chemical biological approach to assess the role of Bm-MPK1 in parasite anti-ROS responses using the synthetic human p38 inhibitor, BIRB796. With this compound we have demonstrated potent inhibition of recombinant Bm-MPK1 *in vitro*. Treatment of adult *B. malayi* parasites and microfilarae with BIRB796, in the presence of ROS, dramatically decreased motility and viability. Using a similar approach with the pyridinylamide JNK inhibitor, N-(4-amino-5-cyano-6-ethoxypyridin-2-yl-dimethoxyphenyl) acetamide, we have also implicated Bm-JNK1 in playing a protective role against ROS. Interfering with the ability of filarial parasites to cope with oxidative stress during infection may offer a new therapeutic approach for treating filarial disease.

Mentor: John Siekierka

Cell Biology and Molecular Medicine

Corey Chang

Dan Li

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Phenotypic Manifestations of Id1 and Id3 Ablation in the Adult Heart

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Congenital heart defects are found in nearly 1% of live human births. Many of these defects are lethal in the absence of surgical intervention. More recently, there is a greater appreciation for the contribution of genetic defects towards cardiac pathology in later stages of development (Moon A 2008). The Id genes (Id1-Id4) play an important role in cardiac development (Lyden D et al 1999). Complete ablation of Id1 and Id3 generates a double knockout (Id dKOs) that is phenotypically lethal at midgestation. Id dKOs exhibit a number of cardiac abnormalities including ventricular septal defects, marked vascular distention and trabecular network abnormalities. While Id genes are expressed in nonmyocardial layers (epicardium, endocardium, endothelium), the affected tissue is the myocardium (Fraidenaich D et al 2004). To study the effects of Id genes on the postnatal heart, we generated a conditional knockout (Id cKOs) in which Id3 is ablated globally while Id1 is ablated via Tie2Cre/loxP recombination, targeting the endocardial/endothelial compartments. While half of the pups die at birth, the other half survive into adulthood and develop a characteristic dilated cardiomyopathy. Adult Id cKO hearts exhibit marked endocardial and perivascular fibrosis. Microarray analysis of Id cKO hearts reveals dysregulation of fibrotic, vascular and hypertrophic markers. Adult Id cKOs also exhibit marked splenomegaly and anemia, suggesting abnormalities in the hematopoietic system (Zhao et al 2010). Furthermore, studies have shown that the Tie2 promoter is active in the hematopoietic compartment, more specifically myeloid lineages (Coffelt SB et al 2010).

We hypothesized that loss of Id in both the hematopoietic and endocardial/endothelial compartments contributes to the observed Id cKO cardiac phenotype. To test this hypothesis, we conducted a series of bone marrow transplantation experiments to determine 1) whether a WT hematopoietic system is capable of rescuing an Id cKO heart from developing dilated cardiomyopathy (correction) and 2) whether an Id cKO hematopoietic system adversely impacts a WT heart (reversion). Preliminary results seem to indicate that transplantation of WT marrow into Id cKO recipients at 2 months of age leads to a reduction in fibrosis, decreased hypertrophy and improved cardiac function compared to Id cKO controls. We are currently investigating phenotypic changes in WT mice transplanted with Id cKO marrow.

Mentor: Diego Fraidenaich

Dan Li

Anti-apoptotic Effect of Proteasome Inhibition – Role of Proteasome Inhibitors in Cardioprotection

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Heart failure is characterized by extensive structural remodeling, including increased apoptosis and cell death. A promising method to prevent heart failure is to reduce loss of functional cardiomyocytes. Proteasome inhibition, previously known for its anti-tumor effects, has been shown to improve cardiac function. In both canine and mouse models of cardiac hypertrophy and ischemic-reperfusion, epoxomicin, a proteasome inhibitor, was found to prevent cardiac remodeling by reducing collagen accumulation and myocyte apoptosis. Based on these observations, we hypothesize that epoxomicin protects cardiomyocytes against the pro-apoptotic stimuli. In this study, we explored the underlying molecular and signaling mechanisms through which epoxomicin prevents apoptosis. Neonatal rat cardiomyocytes were pretreated with 0.01 μ M epoxomicin for 24 hours (shorter treatment did not show protection) and apoptosis was induced by addition of 5 μ M chelerythrine for 30 min. TUNEL staining showed that epoxomicin reduced apoptosis by 50% ($P < 0.05$). This was associated with a significant reduction in cleavage of caspase 3 ($P < 0.01$) and increase in the expression of small heat shock proteins Hsp22 and Hsp25 ($P < 0.05$). Our data also demonstrate that chelerythrine mediated apoptosis was associated with the activation of MEK/ERK pathway and specific phosphorylation of Akt at ser473. Epoxomicin completely abolished the MEK, ERK and Akt phosphorylation. Together our data suggest that 1) epoxomicin prevents the activation of caspase 3 through induction of small heat shock proteins, and 2) chelerythrine induced phosphorylation of MEK, ERK and Akt ser473 may represent a mechanism leading to cell apoptosis, which can be abolished by epoxomicin. Studies on pathways downstream Akt ser473 phosphorylation and the gene expression changes upon epoxomicin treatment are in progress and will identify the molecular mechanisms underlying the cardioprotective effect of epoxomicin. In conclusion, we have demonstrated that proteasome inhibitors can promote cardiac myocyte survival and that proteasome inhibitors can be considered as potential pharmacological candidates to prevent heart failure.

Mentor: Gopal Babu

Dan Shao

Positive feedback regulation between AMP-activated protein kinase and Thioredoxin-1 maintains energy- and redox-homeostasis during myocardial ischemia

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Thioredoxin-1 (Trx1) is upregulated by myocardial ischemia and protects the heart against cell death. We hypothesized that AMP-activated protein kinase (AMPK), a sensor for energy deprivation, plays an essential role in mediating upregulation of Trx1 during ischemia. Expression of Trx1 was increased by prolonged (3h) ischemia (PI) in the mouse heart (3.1 fold, $p < 0.01$ vs sham), an effect which was attenuated in dominant negative (DN)-AMPK mice (Tg-DN-AMPK) (1.0 fold, $p < 0.01$ vs control). Phosphorylation of AMPK was increased by PI (2.2 fold, $p < 0.01$ vs sham) and this was inhibited in DN-Trx1 mice (Tg-DN-Trx1) (1.1 fold, $p < 0.01$ vs control). Thus, AMPK and Trx1 mediate upregulation/activation of one another during PI. The size of myocardial infarction (MI)/area at risk after PI was significantly greater in Tg-DN-AMPK (72% vs 53% $p < 0.05$) and Tg-DN-Trx1 (62% vs 39% $p < 0.05$) than in respective controls, suggesting that both AMPK and Trx1 are protective against PI. Glucose deprivation (GD) upregulated Trx1 in cardiomyocytes (CMs) (2.0 fold, $p < 0.05$), which was accompanied by 2.7 fold activation of AMPK ($p < 0.05$). AICAR, an activator of AMPK, upregulated Trx1 (2.8 fold, $p < 0.05$). Inhibition of AMPK by DN-AMPK attenuated upregulation of Trx1 in response to GD or AICAR (0.6 and 0.5fold, $p < 0.05$). Phosphorylation of AMPK and ACC, a downstream substrate of AMPK, during GD was also significantly attenuated (0.6 and 0.7fold, $p < 0.05$) by downregulation of Trx1. GD-induced cell death was enhanced by inhibition of AMPK (68% vs 55%, $p < 0.05$) or downregulation of Trx1 (60% vs 37% $p < 0.05$). Downregulation of Trx1 inhibited GD-induced Glut4 translocation and glucose uptake (0.3 fold, $p < 0.05$). Phosphorylation of phospho-fructose kinase 2 induced by GD was also attenuated and the increased glycolysis rate (4 fold, $p < 0.05$) was also completely inhibited by sh-Trx1. Decreases in ATP content during GD were enhanced by inhibition of AMPK (1.9 fold, $p < 0.05$) and downregulation of Trx1 (1.5 fold, $p < 0.05$) due to impaired glucose metabolism. These results suggest that AMPK and Trx1 form a positive feedback loop during myocardial ischemia, thereby enhancing each other's function against energy starvation-induced damage to maintain the energy and redox balance in the heart.

Mentor: Junichi Sadoshima

Eman Rashed

Role of Hsp22 in the Mitochondrial Function of Stat3

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Mitochondrial dysfunction due to decreased supply of oxygen, such as during ischemia, leads to impaired ATP generation, which eventually results in cell death. In addition to the well-known mitochondrial proteins directly responsible for oxidative metabolism and ATP production, recent studies have demonstrated that molecules previously not thought to be expressed within mitochondria are actively involved in the regulation of oxidative phosphorylation. The goal of this present proposal is to further define the function of such molecules. Specifically, we will test the role of the heat shock protein Hsp22/H11 Kinase (Hsp22) in the stimulation of mitochondrial respiration by the Signal Transducer and Activator of Transcription-3 (STAT3). Hsp22 is a stress-activated chaperone promoting cardiac cell survival through activation of the Akt pathway, but which is also expressed in mitochondria. STAT3 is a stress-inducible transcription factor essential for cardiac cell survival and growth, which was recently shown to be expressed within mitochondria as well, where it activates oxidative phosphorylation. Our preliminary data demonstrate that manipulation of Hsp22 expression in vivo, both in transgenic and knockout mouse models, modifies both mitochondrial STAT3 distribution and respiration, and that Hsp22 and STAT3 interact in the mitochondria. Based on these observations it is our global hypothesis that Hsp22 stimulates the translocation and activation of STAT3 in the mitochondrial matrix in order to increase mitochondrial respiration upon cardiac stress. Our first goal is to show that Hsp22 translocates to the mitochondria where it activates STAT3, which will be achieved through two Specific Aims. 1. To determine the specific localization of Hsp22 in the mitochondria, and the mechanisms underlying its mitochondrial transfer. 2. To determine in sub-mitochondrial fractions the pattern of Hsp22 protein-protein interactions. Our second goal is to demonstrate that the Hsp22/STAT3 complex increases mitochondrial respiration upon stress, which will also be achieved through two Specific Aims. 1. To determine the mechanisms of stress-activated STAT3 phosphorylation by Hsp22. 2. To determine whether deletion of Hsp22 impairs the adaptation of oxidative phosphorylation to stress upon pressure overload. Unraveling the activation of novel signaling pathways between Hsp22 and STAT3 may be of clinical relevance for patients exposed to chronic overload or ischemia, and at risk of irreversible cardiac damage.

Mentor: Christophe Depre

Jessica Toli

Differential Sequestration of mRNA in Ribonucleoprotein Granules in the Heart

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Posttranscriptional regulation of gene expression is fundamental to organogenesis and pathogenesis. One of the modes of regulation that has been observed, particularly during stress conditions, is the formation of stress granules. These granules have been shown to sequester mRNA in a microscopically visible complex with RNA-binding proteins that mainly include cytotoxic granule-associated RNA binding protein 1 (TIA-1 and TIAR). The proposed functions of these granules range from RNA storage to mRNA decay, depending on the cell and its environment. Until now we don't know if these granules exist in the myocardium under any condition. Accordingly, we hypothesized that RNA granules assemble in the heart in association with selective mRNAs as a mechanism to differentially regulate protein translation during myocyte growth or stress conditions. To address this, we applied immunocytochemistry and immunoprecipitation (IP)-qPCR approaches in cardiac myocytes exposed to growth, hypoxia, and hypoglycemic conditions, using a TIA-1/TIAR antibody. For the first time, our results show an abundant formation of RNA granules in cardiac myocytes maintained in complete growth medium. However, these structures rapidly disappear (>90%) upon serum, glucose, or oxygen deprivation, suggesting a possible role in releasing stored mRNA that may be required under stress conditions. Similarly, we detected abundant TIA-1-positive granules in the adult mouse heart that appeared to increase after transverse aortic constriction for 7 days (more samples needed to determine significance). IP-qPCR established that the granules harbor selective mRNA molecules that included myosin heavy chain beta, sprouty2, AKT, and hypoxia-inducible factor-1 alpha, but excluded Cdk7, EIF4E, transcription factor IIB, and TATA-binding protein. Presently, a more comprehensive quantitative analysis of the RNA associated with the granules in control vs. hypertrophied hearts is underway using extensive deep RNA sequencing technology. Thus, our results uncover the assembly of unique ribonucleoprotein granules in the heart that harbor selective RNA molecules that may play a role in posttranscriptional regulation of gene expression in the heart during health and disease.

Mentor: Maha Abdellatif

Joel Schneider

Molecular Mechanisms of Cardiomyopathy in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is an x-linked recessive disorder that affects 1:3500 males. The disease is characterized by the absence of the dystrophin protein, leading to severe muscle degeneration. Additionally, due to the absence of Dystrophin, key proteins necessary for cell sarcolemmal stability and cell signaling are no longer localized properly and cannot function. The mouse model of DMD is the mdx mouse and recapitulates the absence of Dystrophin in contractile muscle. By injecting wild-type embryonic stem cells into blastocysts prone to develop muscular dystrophy, our lab is able to rescue the dystrophic phenotype and study the mechanisms by which the rescue occurs. This includes the re-localization of proteins such as neuronal Nitric Oxide Synthase (NOS1) to their appropriate, wild-type compartments, thereby restoring the cells to a wild-type morphology. We have quantified differences in nitric oxide production in mdx cardiac myocytes isolated via Langendorff perfusion using a nitric oxide responsive dye and identified variances in nitric oxide synthase protein expression via western blot and immunofluorescence.

To study mechanisms of mdx in-vitro, we have also generated mouse induced pluripotent stem cells (miPSCs) that are from mdx mice. Using the original Yamanaka protocol, we used retroviral infection to deliver Oct3/4, Sox2, c-Myc and Klf4 to mdx-mouse embryonic fibroblasts. Upon infection, we isolated embryonic stem cell like (ESC) colonies based on morphology alone. RT-PCR analysis of isolated colonies showed positive expression of the 4 pluripotency associated markers as well as positivity for endogenously expressed Nanog. These cells also stained positively for alkaline phosphatase activity. To study the cardiac expression of dystrophin, we have differentiated these cells as well as mdx (+/-) mESCs into autonomously beating cardiac myocytes in culture. Using the embryoid body technique. The derived myocyte populations beat for up to 3 weeks in culture and stained positively for troponinT and alpha-actinin. The contractility of the myocytes derived can be quantified via calcium flux measurements and modified by the addition of agonists. These myocytes also express multiple isoforms of nitric oxide synthase. Taken together, these experiments present an additional mechanistic target for which treatments for DMD can be approached.

Mentor: Diego Fraidennaich

Joseph Vitale

The Pluripotent Stem Cell Rescue of Muscular Dystrophy

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We injected WT mouse pluripotent stem cells (PSCs), (induced pluripotent stem cells or iPSCs and embryonic stem cells or ESCs), into mdx and mdx:utrophin mutant blastocysts, which are predisposed to develop Duchenne muscular dystrophy (DMD). Mdx:utrophin mice suffer from a much more severe form of DMD than mdx. We also injected WT PSCs into sarcoglycan- δ (SG δ KO blastocysts, which are predisposed to develop limb-girdle muscular dystrophy-2F (LGMD-2F). In mdx chimeras, PSC-dystrophin was supplied to the muscle sarcolemma to effect corrections at morphological and functional levels with a low rate of PSC incorporation (10-30%). In the mdx:utrophin mutant chimeras, although PSC-dystrophin was also supplied to the muscle sarcolemma, mice still displayed poor skeletal muscle histopathology and kyphosis. In the SG δ chimeras, PSC-SG δ was supplied to the muscle sarcolemma. Unlike mdx chimeras, the SG δ chimeras needed at least 60% PSC incorporation for rescue of the heart, pectoralis and quadriceps. However, the diaphragm is not rescued with 60% chimerism and also suffers from diminished levels of dystrophin along with no upregulation of compensatory utrophin.

In conclusion, not every muscular dystrophy paradigm can be rescued with PSC treatment.

Mentor: Diego Fraidenraich

Lo Lai

Type 5 Adenylyl Cyclase Disruption Improve Exercise Tolerance via Mitochondria Biogenesis

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Adenylyl cyclase type 5 knockout (AC5 KO) mice have been reported as a new longevity model, which mimics caloric restriction (CR). In contrast to CR, AC5 KO mice eat more than WT, but weigh less. Several CR associated studies in humans demonstrated that long term CR may induce lean muscle loss, thus decrease exercise capacity. We tested our AC5 KO mice for their skeletal muscle phenotype. Surprisingly, AC5 KO mice maintain a similar muscle mass with WT, but contain richer oxidative slow-twitch fibers. We further determined that AC5 KO mice had enhanced exercise capacity. cDNA microarray revealed genes in skeletal muscle of AC5 KO mice, which are mainly involved in mitochondrial oxidative metabolism. Enhanced mitochondrial oxidative capacity and biogenesis in skeletal muscle was confirmed, $p < 0.05$, by increased cytochrome C oxidase activity (50%), ATP content (25%), citrate synthase activity (35%), and level of PGC-1 α (1-fold). Thus, AC5 inhibition may be a novel approach to prevent obesity and diabetes, while increasing exercise capacity through improved mitochondrial function in skeletal muscle.

Mentor: Dorothy Vatner

Mingyue Han

Gata4 Expression Is Regulated, Primarily, Via A Phospholipase C Beta1-miR-26-dependent Posttranscriptional Mechanism During Cardiac Hypertrophy

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GATA4 plays an essential role in the development of cardiac hypertrophy, however, its mode of regulation remains unknown. In this study we investigated transcriptional vs. posttranscriptional mechanisms that are involved in regulating GATA4 in the heart during neonatal and pressure-induced hypertrophic growth. Our data show that GATA4 gene translation is significantly higher during neonatal (6.8 ± 1 fold) and pressure overload-induced (2.9 ± 0.4 fold) cardiac hypertrophy vs. a sham-operated or normal adult mouse heart, compared to its transcription, as measured by RNA polymerase II chromatin immunoprecipitation-deep sequencing (2 and 0.97 fold, respectively), or mRNA levels (1.6 ± 0.4 and 1.1 ± 0.15 fold, respectively), which suggests mainly a posttranscriptional mode of regulation during pressure-induced hypertrophy. The 3'UTR of GATA4 harbors conserved miRNA target sites. Of those, only miR-26 inversely correlates with GATA4 expression during neonatal and pressure overload-induced hypertrophy. Indeed, deletion of the miR-26 site results in $\sim 2x$ increase in GATA4 protein in cardiac myocytes, whereas modulation of the miR-26 levels inversely impacts GATA4 levels. In addition, supplying cardiac myocytes with exogenous miR-26 inhibits GATA4-dependent transcription, endothelin-induced hypertrophy, and sensitizes the cells to apoptotic insults. We also found that miR-26 targets phospholipase C-beta1 (PLCb1), which through a negative feedback loop inhibits the expression of miR-26. Ultimately, we questioned whether reducing miR-26 in the adult heart is indeed sufficient for inducing upregulation of GATA4 and PLCb1. For that purpose we injected locked-nucleic acid-modified antisense miR-26a intravenously. This treatment resulted in very effective downregulation of miR-26, which was accompanied by the upregulation of both its targets within 7 days to the same extent observed during hypertrophy. Although, this was not accompanied by an increase in heart/body weight at this time point, it did induced upregulation of, at least, atrial natriuretic factor and skeletal-alpha-actin. From these results we conclude that GATA4 expression is mainly regulated by a PLCb1-miR-26-dependent mechanism during pathological hypertrophy.

Mentor: MAHA ABDELLATIF

Smita Shukla

Post Transcriptional Regulation of Phospholipase-C β 2 (PLC β 2) by MicroRNAs

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Macrophages, on activation with certain exogenous and endogenous stimuli, modulate their expression of cytokines and growth factors and play roles in the regulation of inflammation, wound healing, innate and acquired immunity. Macrophages can be 'classically activated' (M1) or 'alternatively activated' (M2a) and the cytokines and growth factors that they produce vary depending on the particular stimulators. M1 activation is induced by stimulation with IFN gamma and/or TLR agonists such as LPS, is pro-inflammatory and characterized by the expression of IL-1, IL-6, IL-12, TNF alpha and iNOS. M2a activation is anti inflammatory, and is induced by IL-4 or IL-13 through the IL-4R α . M2 activation is characterized by elevated expression of TGF- β and IL-10. Studies from our lab have described an IL-4 and IL-13 independent mode of converting M1 to M2-like macrophages that is induced by the metabolite adenosine and requires the adenosine A2A and A2B receptors (A2AR and A2BR). We have termed this as the M2d phenotype. We recently found that LPS rapidly and specifically suppresses PLC beta 2 (PLC β 2) expression at the post transcriptional level by destabilizing its mRNA, and that this suppression plays a role in the A2AR receptor mediated switch of macrophages from an inflammatory(M1) to an angiogenic(M2d) phenotype. The mechanism of destabilization of PLC β 2 mRNA, however, is not yet known. This study aims to elucidate this mechanism of destabilization PLC β 2 mRNA. Analysis of the role of the PLC β 2 3'UTR using reporter assays suggests a role in this destabilization. Microarray profiling of microRNAs modulated in macrophages by LPS and NECA (a non-specific adenosine receptor agonist) indicated several microRNAs that are regulated by these treatments. These candidate microRNAs will be individually knocked down or over-expressed to determine if they play a role in the destabilization of PLC β 2 mRNA. Finally, the effects of over-expression or knockdown of these microRNAs will be studied with regard to their modulation of the cytokines typical of M1 and M2 phenotypes. For M1 activation, we will study TNF α and IL-12; for M2 activation, we will study VEGF and IL-10. In addition, the role of RNA binding proteins such as Tristetraprolin and HuR that are involved in mRNA stabilization is also being studied.

Mentor: S J Leibovich

Xin Zhao

Cardiac-specific Overexpression of the β 1A-Adrenergic Receptor in Rats: a Model of Enhanced Cardiac Contractility and Autonomically Decreased Heart Rate

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We examined cardiac function in a novel TG rat model of cardiac specific overexpression of β 1A-adrenergic receptors (β 1A-ARs). Left ventricular (LV) function was studied in anesthetized TG rats and wild type littermates (NTLs), and heart rate (HR) regulation by telemetry in conscious rats. Cardiac hemodynamics, determined using micromanometry (LV pressure and dP/dt) and echocardiography (ejection fraction, EF), revealed enhanced contractility in TG rats (n=6) vs NTLs (n=7) (+dP/dt: 9752 ± 159 vs 8067 ± 306 mmHg/s, $p < 0.01$; EF: $87 \pm 1.2\%$ vs $74 \pm 0.7\%$, $p < 0.01$, respectively), where as LV systolic pressure was not different (122 ± 5 vs 124 ± 6 mmHg, respectively). HR, however, was significantly lower in TG rats vs NTLs (293 ± 5 vs 327 ± 10 bpm, $p < 0.05$), due both to decreased sympathetic drive (diminished HR fall to β -AR blockade with propranolol, 5 mg/kg: -3 ± 1 vs -11 ± 2 bpm, $p < 0.05$, respectively) and enhanced parasympathetic tone (increased HR rise to muscarinic receptor blockade with atropine, 4 mg/kg: $+35 \pm 3$ vs $+23 \pm 2$ bpm, $p < 0.05$, respectively). Thus, cardiac β 1A-ARs overexpression in the rat results in a novel model of enhanced LV contractility and autonomically decreased heart rate.

Mentor: Stephen F. Vatner

Yanfei Yang

Mir-206, Distinct from Mir-1, Mediates YAP-Induced Cardiac Hypertrophy and Survival

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The *Drosophila* Hippo regulates apoptosis and proliferation through suppression of Yorkie, a transcription factor co-factor, and the microRNA, bantam. YAP, a homologue of Yorkie, stimulates hypertrophy and inhibits apoptosis in cardiomyocytes (CMs). We found that miR-206 is significantly upregulated by YAP in CMs. miR-206 and miR-1 are homologous muscle-specific microRNAs, constituting the miRNA-1/206 family. YAP-induced upregulation of miR-206 was confirmed with Northern blot (+1.4 fold, $p < 0.05$), qRT-PCR (+1.6 fold, $p < 0.05$), and a reporter gene containing the miR-206 enhancer (+1.9 fold, $p < 0.01$). Upregulation of miR-1 or miR-1 enhancer by YAP was not significant. miR-206 induced hypertrophy, as evidenced by increases in cell size (CS, +1.2 fold, $p < 0.05$), total protein content (PC, +1.1 fold, $p < 0.05$), and mRNA expression of atrial natriuretic factor (ANF) (+1.8 fold, $p < 0.05$) in CMs. However, miR-1 inhibited hypertrophy, as evidenced by decreases in PC (-10%, $p < 0.05$). Downregulation of miR-206 attenuated YAP-induced hypertrophy, as evidenced by decreases in CS (-15%, $p < 0.05$), PC (-10%, $p < 0.05$) and ANF luciferase activity (-64.4%, $p < 0.05$). However, downregulation of miR-1 tended to exaggerate YAP-induced hypertrophy. We generated transgenic mice (Tg) with cardiac specific overexpression of miR-206 (Tg-miR-206). Tg-miR-206 exhibited cardiac hypertrophy at baseline, as evidenced by increases in left ventricular weight/body weight (+1.3 fold, $p < 0.05$), CM cross sectional area (+1.3 fold, $p < 0.01$), and mRNA expression of ANF (+2.9 fold, $p < 0.01$). Both YAP and miR-206 were downregulated after myocardial infarction (MI) in the heart in vivo. In contrast with miR-1, which induces cell death, overexpression of either YAP or miR-206 increased survival and downregulation of miR-206 decreased survival of CMs in vitro (+1.4 and +1.2 fold, -38%, $p < 0.05$). We also generated Tg with cardiac specific overexpression of anti-sense miR-206. These mice exhibited a larger MI in response to ischemia/reperfusion (I/R) than control mice, suggesting that endogenous miR-206 is protective during I/R. In summary, YAP upregulates miR-206, which in turn mediates hypertrophy and survival in CMs. miR-206, distinct from miR-1, mimics the function of *Drosophila* bantam.

Mentor: Junichi Sadoshima

Department of Medicine

Andrew Marple

Jessian Munoz

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Investigating the Impact of Helminth Co-Infection on CD8 T Cell Immunity

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The protozoan parasite *Toxoplasma gondii* induces a strongly polarized Th1 cytokine response upon infection, resulting in the production of IFN-gamma by CD8+ T cells, which is essential for protective immunity. Conversely, infection with an intestinal nematode results in the generation and systemic dissemination of Th2 cytokines. Numerous studies have shown that helminth co-infection can attenuate immune responses to intracellular pathogens and inhibit the proper development of Th1 responses. Clinically this is significant during vaccinations involving helminth-infected individuals, where the efficacy of vaccination is decreased. It has been previously reported in a murine model of co-infection with *T. gondii* and *Heligmosomoides polygyrus* that both acute and chronic CD8+ T cell responses against *T. gondii* were suppressed in helminth co-infected mice, thus suggesting a negative impact of co-infection on the CD8+ T cell response. However, precisely how co-infection impinges upon the priming and differentiation of antigen-specific CD8+ lymphocytes remains to be determined.

Using a novel Kb-peptide tetramer binding assay for Tgd057 reactive CD8 T cells, here we show that mice infected with *H. polygyrus* and subsequently vaccinated with a non-replicative strain of *T. gondii* are capable of developing an antigen-specific response to *T. gondii*. However, this response is diminished compared to challenge with *T. gondii* alone. Co-infected mice develop fewer antigen-specific CD8+ T cells, a decreased fraction of effector differentiated cells, and exhibit reduced production of IFN-gamma in response to both peptide and whole parasite restimulation. When compared to effector cells from *T. gondii* only infected mice, effector cells from co-infected mice retain an attenuated phenotype suggesting an intrinsic defect in CD8+ T cell function. This phenotype is lost when helminth infection is cleared before *T. gondii* vaccination, indicating a direct effect of the helminth on the proper priming of the CD8+ T cell priming and differentiation. These results indicate that, rather than inhibiting CD8 priming itself, the principal effect of helminth infection is to attenuate the differentiation of effector lymphocytes. Current experiments using large-scale RNA sequencing techniques are aimed at elucidating how helminth infection alters the differentiation program of the effector cell lineage and will pinpoint upstream mechanisms resulting in this attenuated response.

Mentor: George Yap

Jessian Munoz

Post Transcriptional Regulation of the Sonic Hedgehog Pathway: Key to Human Glioblastoma Cancer Stem Cell (CSC) Chemoresistance

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Glioblastoma Multiforme (GBM) is the most common adult intracranial malignancy, it is also the most aggressive with a ~11-month median survival rate. Radio/Chemoresistance is a common feature of GBM and recent evidence has shown that these conventional methods of neoplastic treatment spare a small subset of CD133+ GBM cells, characterized as GBM Cancer Stem Cells (CSCs). Sonic Hedgehog (SHH) signaling has been shown to be constitutively active in CD133+ GBM CSCs. GBM extracellular stromal cells secrete SHH in a paracrine manner, the SHH cell surface receptor, Patched-1 (PTCH1), is required for GBM intracellular activation. Downstream targets of SHH signaling include CyclinD, PTCH1 and the transcriptional activator Gli1. MicroRNA (miR) are small RNA molecules, which negatively regulate mRNA translation by binding to the 3' untranslated region (UTR), which results in target mRNA degradation. Although limited, miR profiles of GBM CSC and GBM non-CSCs have revealed differential expression, including down-regulation of miR-9, -34a, -124, and 137. Here we show that the miR-9 decreased in GBM CSCs results in a heightened response to extracellular SHH mitogen, promoting the chemoresistance and survival of GBM cells. We present preliminary data showing miRNA regulation of PTCH1, loss of this inhibition results in membrane over-expression of functional PTCH1 protein. First we modeled this mechanism in PTCH1 deficient and candidate miR deficient cell lines. Utilization of precursor miR (pre-miR) and antagomiR (anti-miR) as well as a Luciferase construct expressing the PTCH1 3' UTR showed downregulation of endogenous PTCH1 and 3'UTR specificity. Once PTCH1 miR regulation was confirmed to be functional, GBM CD133+ CSCs were selected by fluorescent activated cell sorting (FACS). The same methods previously described will be used to characterize miR dysregulation of hedgehog PTCH1 receptor. Also evaluated were GBM radioresistant and chemoresistant properties and the ablation of these by the introduction of targeted pre-miR pre-treatment. In addition the role of the stem cell protein Re-1 silencing transcription factor/Neural Restrictive Silencing Factor (REST/NRSF) was evaluated as an initiating step in the generation of CD133+ GBM CSCs. To this end, CD133- GBM cells were transfected with a REST overexpression vector as CD133+ GBM CSCs were transfected with a REST siRNA vector, both were evaluated for cancer stem cell properties and oncogenic capacities. These experiments will be repeated in patient GBM samples to show the translational implications and novel treatment approach obtained by better understanding the regulatory mechanisms of the SHH pathway.

Mentor: Pranela Rameshwar

Priyanka Singh

Modulation of the microglia/macrophage response in the spinal cord by a Toll-like receptor 9 (TLR9) agonist

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Toll-like receptors are expressed in effector cells of the innate and adaptive immune systems and have been implicated in the induction of immune responses in the central nervous system (CNS). Toll-like receptor 9 (TRL9), in particular, is predominantly expressed in microglia, enabling them to sense invading pathogens and cell damage. As part of the innate immune system intrinsic to the CNS, microglia are first-responders to injury, reflected in their proliferation, altered morphology and effector molecule expression upon cell activation. This investigation was undertaken to determine whether the intrathecal administration of the TLR9 agonist, cytosine-phosphate-guanine oligodeoxynucleotide 1826 (CpG ODN1826), induces microglial activation in the spinal cord. The microglial response to CpG ODN1826 was assessed by immunohistochemistry and flow cytometry.

CpG ODN1826 induced changes in both the intensity of the immunoreactivity to Iba-1, a marker of microglia, and cell morphology, as compared to untreated controls. Iba-1 staining was generally higher in the caudal end of the spinal cord than in the rostral end. More microglia displayed activated/reactive morphology with round cell bodies, thickened and retracted processes or no processes, towards the caudal end of the spinal cord. In contrast, more microglia displayed a morphology associated with a resting/surveillance state, with long, ramified processes and smaller cell bodies, towards the rostral end of the cord. The observed changes in microglia morphology reflects their transition from a resting/surveillance state to an easily discernable activated/reactive state in response to CpG ODN1826, but also suggest that microglial activation is not uniform throughout the various levels of the spinal cord when the agonist is given intrathecally. The upregulation of Iba-1 intensity is also indicative of microglial activation. In addition, CpG ODN1826 increased CD11b- (microglia/macrophages) and CD45- (cells of myeloid origin) positive cell number as assessed by flow cytometry. It remains to be determined whether the increase in CD11b- and CD45-positive cell number is due to proliferation or chemotaxis.

Mentor: Robert F. Heary and Stella Elkabes

Veronika Khariv

Molecular anomalies in the central nervous system of the Toll-like receptor 9 knockout mice

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Mammalian Toll-like receptors (TLRs) share similarities with the Toll protein which has been implicated in the development of the central nervous system (CNS) in *Drosophila Melanogaster*. Although TLRs are best known for the induction of innate immune system in response to pathogens, increasing evidence supports the notion that they play additional intriguing roles in the CNS including sterile inflammation and development.

Using the TLR9 knockout mice as a model, we investigated whether disruption of TLR9 affects CNS function. Earlier studies in our laboratory indicated that TLR9 knockout mice exhibit sensory and motor deficits potentially attributable to spinal cord and cerebellar dysfunction (Goodus et al, 2011, Abstracts, Society for Neuroscience, 41st Annual Meeting). Here we investigated whether these behavioral deficits are also paralleled by molecular abnormalities. We focused on the expression of plasma membrane calcium ATPase 2 (PMCA2) in the brain and spinal cord, as we previously showed that this calcium pump is important for the function and survival of neurons in the spinal cord and the cerebellum. In addition, studies from other investigators indicated that PMCA2 is critical for hippocampal function.

We report that PMCA2 protein levels are significantly decreased in the spinal cord and the hippocampus of the TLR9 knockout as compared to the wild type controls. In contrast, there were no changes in PMCA2 protein expression in the cerebellum suggesting regional specificity of the changes observed. The relationship between TLR9 and PMCA2, the molecular and cellular mechanisms underlying the decrease in PMCA2 in the spinal cord and hippocampus of TLR9 knockout mice and the functional and developmental significance of the changes observed will be the focus of future investigations.

Mentor: Robert Heary and Stella Elkabes

Neuroscience

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IGF2 promotes murine neural stem/progenitor cell growth but is dispensable for murine ES cell self-renewal.

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Recent studies have provided evidence that the insulin-like growth factors regulate the growth of embryonic and adult stem cells. Based on their different binding affinities to the IGF-1R and the insulin receptor (IR) we hypothesized that IGF2 would exert distinct effects from IGF-1 on both murine ES cells and adult neural stem/progenitor cell growth and self-renewal. Indeed, IGF2 promoted neural stem/progenitor cell expansion better than either IGF-1 or standard culture medium (containing superphysiological levels of insulin). A combination of IGF1 and IGF2 mimicked standard neurosphere growth conditions in terms of neurosphere number and size; however, limiting dilution and differentiation analyses revealed that IGF2 was superior to IGF-1 in promoting neurosphere number. Knockdown of either the IR or IGF-1R using shRNAs supported the conclusion that the IGF-1R promotes progenitor proliferation whereas the IR is important for self-renewal. RT Q-PCR revealed that IGF2 increased Oct4, Sox1 and FABP7 mRNA levels in neurosphere cells. However, when murine ES cells were maintained in serum free, feeder free conditions, colonies formed in the absence of insulin. Furthermore, the ES cells could be passaged multiple times without insulin, IGF2 or IGF-1. Furthermore, neither the expression of Oct4 nor nanog depended on insulin/IGF in the medium, and there were no differences in alkaline phosphatase staining. Altogether our data support the conclusion that IGF2 promotes the self-renewal of neural stem/progenitors via the IR. By contrast, IGF-1R functions as a mitogenic receptor that increases cell cycle progression of progenitors. Surprisingly, IGF system activation is not required for murine ES cell maintenance or self-renewal. Supported by a Dean's Grant from NJMS awarded to SWL and TLW, R21HL094905 awarded to DF and F31NS065607 awarded to ANZ.

Mentor: Steve Levison

Hongxin Chen

Effects of Ionizing Radiation on Neural Precursor Cells

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Damage to normal human brain cells from targeted exposure to ionizing radiation may occur during the course of cancer radiotherapy or from accidental exposure. Delayed effects may complicate the acute immediate effects and in particular have been associated with neurodegeneration and cognitive decline. These delayed effects may arise from the slow death of irradiated cells, damage to cells that survive the radiation exposure, or from negative effects on normal cell replenishment non-targeted brain cells from neural stem cells. Using quantitative analytical approaches, the goal of this proposal is to elucidate cellular and molecular changes associated with the exposure of neural stem cells (NSCs) to high and low dose of ionizing radiation. Subventricular zone (SVZ) neural precursors isolated from newborn mouse pups were analyzed for survival, proliferation and differentiation after exposure to low and high doses of γ rays. Surprisingly, the number of neurospheres formed showed no significant change from control to 8 Gy of γ irradiation. Meanwhile, the size of spheres increased slightly at low dose (0.4 or 0.5 Gy, no significant change) and decreased dramatically at 8 Gy ($p < 0.001$). Cell cycle analysis showed S-phase cells decreased by more than 50% percent at 8 Gy and there was a G2/M phase arrest compared to untreated cells at both 24 hour and 48 hours after irradiation. We also differentiated neurospheres 8 days after irradiation and assessed the formation of astroglia, neurons and oligodendrocytes. However, there was no significant change between control cells and irradiated cells ($P > 0.05$). These data suggest that neural precursor cells are resistant to ionizing irradiation, but that their proliferation is inhibited subsequent to exposure to ionizing irradiation.

Mentor: Steve Levison

Jessica Magid-Bernstein

Understanding Treg heterogeneity: the key to unraveling autoimmunity?

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T regulatory cells (Tregs) are important in the development and progression of autoimmunity. Tregs are usually identified by their expression of CD25 and Foxp3, although functionally and phenotypically diverse subsets exist. By identifying various subsets we hope to clarify their function and role in autoimmunity.

To identify human Treg subsets we analyzed expression of CD27, CD127, CD39, and CTLA4 on CD4 T cells with varying CD25 levels. A CD27⁺CD127⁻ phenotype correlates highly with CD25 and Foxp3 expression. CD39 expression is higher on CD4⁺CD25^{hi} T cells than on CD25^{med/lo} cells. We cultured sorted Treg subsets with T effector (Teff) cells to measure suppression of proliferation and cytokine production. High expression of CD25 was a better marker for suppressive ability than the CD27⁺CD127⁻ phenotype. CD39⁺ Tregs did not suppress Teff cell proliferation when activated with anti-CD3 and anti-CD28, but proliferation was suppressed when cells were cultured in the presence of anti-CD3 and irradiated allogeneic antigen presenting cells (APCs). The ability of APCs, but not recombinant anti-CD28 antibody, to bind with the negative costimulatory molecule CTLA4 on these Tregs may account for their differential suppressive abilities between these two culture conditions. Elucidating Treg mechanisms and subsets will enable us to clarify how Tregs suppress, how they are defective in autoimmunity, and ultimately how this defect can be overcome to treat autoimmunity.

Mentor: Christine Rohowsky-Kochan

Kellie Janke

Infusion of hippocampal BDNF slows acquisition of delay eyeblink conditioning in the Wistar-Kyoto but not Sprague Dawley rat.

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Brain-derived neurotrophic factor (BDNF) and its high affinity receptor, trkB, are extensively expressed in the mammalian hippocampus, and are necessary for neuronal development, survival, and synaptic plasticity associated with learning and memory. Mood disorders, including anxiety and post-traumatic stress disorder (PTSD), have been linked to reductions in hippocampal BDNF, hippocampal size, and impairments in hippocampal-dependent learning. Conversely, individuals with anxiety disorders exhibit accelerated learning in a classically conditioned eyeblink response using a delay paradigm (DEBC). While the hippocampus is not necessary to acquire DEBC, hippocampal lesions accelerate learning in this task. Given that accelerated learning of DEBC may signal anxiety vulnerability and hippocampal dysfunction, we have been investigating the role of hippocampal BDNF on DEBC acquisition in an animal model of anxiety vulnerability. Wistar-Kyoto rat (WKY), is stress sensitive, demonstrates behavioral inhibition, has reduced hippocampal BDNF, and exhibits accelerated acquisition of DEBC compared to a control strain, the Sprague Dawley rat (SD). In the present study, WKY and SD rats were tested in the open-field prior to DEBC training to assess anxiety-like behavior and divided into BDNF or saline infusion groups. Infusions were given bilaterally into the dentate gyrus of the hippocampus one hour prior to training on days one and two of conditioning. The conditioning consisted of 100 trials per day with a 500ms conditioned stimulus (CS, 82dB white noise) co-terminating with a 10ms unconditioned stimulus (US, 10V stimulation) and an average inter-trial interval (ITI) of 30s. A third day of training was used to evaluate extinction in which the US was removed after the first 40 trials. Twenty-four hours after extinction training animals were sacrificed and brain tissue excised and stored for BDNF protein quantification. WKY rats treated with saline were significantly faster at acquiring a conditioned eyeblink response and slower to extinguish compared to the SD. Importantly, BDNF infusion significantly dampened the learning of the WKY rats. These results suggest that hippocampal dysfunction may play a role in the learning bias seen in the WKY rat and that enhancing synaptic plasticity may normalize learning in an anxiety vulnerable strain.

Mentor: Kevin Pang

Lisamarie Moore

Spheroids: an in vitro model to study quaterpotential, PDGF $\alpha\alpha$ responsive, progenitors of the rat subventricular zone

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The subventricular zone (SVZ) contains neural stem cells and progenitors of various potentialities. Although initially parsed into A, B, and C cells, it is clear that the population of cells that comprise this germinal zone is significantly more diverse. The aim of this study was to characterize the developmental potentials of the platelet derived growth factor- $\alpha\alpha$ (PDGF $\alpha\alpha$) responsive cells of the SVZ. When grown in 30% B104 neuroblastoma cell line conditioned hormone supplemented medium or 10ng/ml PDGF $\alpha\alpha$, dissociated neonatal rat SVZ cells divide to produce non-adherent clusters of progeny we refer to as spheroids. Upon differentiation these spheroids develop into neurons, type 1 astrocytes and oligodendrocytes. When maintained in media supplemented with 10ng/ml BMP-4 or 20% fetal bovine serum they also produced type 2 astrocytes. Under specific in vitro conditions, one spheroid could produce neurons, oligodendrocytes, type 1 and type 2 astrocytes. Spheroids likewise could be generated from the neocortex. Newborn neocortex spheroids differentiated similar to those from the SVZ. However, spheroid production from the adult cortex was less than 20% of that obtained from the adult SVZ and spheroids from the adult cortex only produced glial cells. By contrast, SVZ spheroid producing capacity diminished only slightly from birth to adulthood and there was no change in their potentiality. Altogether these data demonstrate that there are PDGF $\alpha\alpha$ responsive progenitors that reside in the SVZ throughout the lifespan. These precursors are quaterpotential, competent to produce neurons, oligodendrocytes, type 1 and type 2 astrocytes.

Mentor: Steven W. Levison

Meghan Davis Caulfield

**Facilitated acquisition of eyeblink conditioning in those at risk for anxiety disorders:
Concordance among scales of inhibited temperament**

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Behavioral inhibition (BI) is a risk factor for anxiety disorders typified by extreme withdrawal in the face of novel social and nonsocial challenges. To the extent that anxiety disorders have a core feature of avoidance, associative learning processes play a central role in the development and expression of anxiety disorders. Previous work has shown that those classified as behaviorally inhibited by scales developed by Gladstone & Parker (Adult and Retrospective Measures Behavioural Inhibition, AMBI and RMBI, respectively) acquire an eyeblink conditioned response faster during delay-type conditioning (500ms, 1000-Hz tone conditioned stimulus (CS) co-terminating with a 50-ms airpuff unconditional stimulus (US)). Here, we have two primary questions: 1) do published measures of BI differ in their sensitivity to differentiate facilitated acquisition? 2) are these individual differences also apparent in activity of the cerebellum and orbital frontal cortex in response to presentations of novel stimuli as measured during functional magnetic imaging (fMRI)? College students (ages 18-23 years old) participated for research credit or \$10.00 per hour. Of 126 students who thus far participated usable conditioning data was available for 80 (46 were excluded poor signal quality, inability to stay awake for the duration of the required 1 h, equipment difficulties, or lack of an unconditioned response (UR)). Training consisted of 45 paired CS-US trials immediately followed by an extinction period (15 CS alone trials). All participants completed the AMBI and RMBI, as well as the Reznick Concurrent and Retrospective Self-Report of Inhibition (CSRI and RSRI) and the Spielberger State/Trait Anxiety Inventory (STAI). Preliminary data shows a strong concordance between the AMBI and CSRI ($r^2=.625$), the RMBI and RSRI ($r^2 = .661$) as well as all four correlating highly with the STAI-Trait, but not as well with the STAI-State. Replicating earlier work, facilitated acquisition is apparent at the extremes of the scales, representing those at risk for developing anxiety disorders. Preliminary analyses of the imaging data suggest that cerebellar activity is present when viewing novel vs. familiar face and scene stimuli. Specifically, while widespread cerebellar activation was present for the scene stimuli, face stimuli more specifically activated lobule VII, a region of the cerebellum suggested to be related to salience detection. These preliminary data support a learning-diathesis model for the development of anxiety disorders.

Mentor: Richard Servatius

Miranda Johnson

Amylin Administration as a Potential Intervention Strategy for Improving Leptin Sensitivity in the DIO Neonate

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Selectively bred diet-induced obese (DIO) rats overeat and rapidly become obese when fed a high-energy (HE) 31% fat, 25% sucrose diet compared to diet-resistant (DR) rats. Our lab has demonstrated that DIO rats have inherent leptin resistance as evidenced by a decreased response to the anorectic and thermogenic effects of leptin. In addition, DIO rats have reduced hypothalamic leptin receptor binding and downstream signaling, as measured by reduced neuronal pSTAT3 expression prior to becoming obese. Development of hypothalamic pathways involved in energy balance regulation occurs during the first 2wks of life and is leptin-dependent. Intervention strategies such as large litter rearing, applied during this developmental period, increase leptin receptor binding and axonal outgrowth in arcuate nucleus (ARC) neurons in association with reduced adiposity when placed on an HE diet as an adult. In lean rats, exogenous administration of amylin, an anorexigenic peptide co-secreted with insulin, increases leptin-induced pSTAT3 and decreases food intake and body weight gain. Amylin and leptin co-administration increases leptin receptor binding in the ventromedial nucleus (VMN) and ARC in lean rats. When applied to the DIO model, amylin and leptin co-administration restores leptin responsiveness by reducing food intake and adiposity when fed a high fat diet. Taken together, amylin may increase leptin responsiveness in the adult DIO rat by increasing leptin receptor binding and signaling. Here, we show that DIO rats have reduced amylin receptor binding in the dorsomedial portion of the VMN, a leptin-receptor positive region, compared to DR rats. These data lead to the hypothesis that early amylin administration to neonates will improve leptin sensitivity during the leptin-dependent hypothalamic growth period for later prevention of obesity as an adult.

Mentor: Barry E. Levin

Nolan Skop

Engineering a Microscaffold to Improve Neural Stem Cell Transplantation for Traumatic Brain Injury Repair.

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Regenerating the central nervous system has become more feasible with the growing field of stem cell therapies. However, stem cells injected into a cystic cavity are limited in regenerative potential due to the lack of a supportive matrix. An injectable scaffold made of multifunctional chitosan microparticles can serve as a structural and functional support for cells transplanted after TBI. The goal of this study was to optimize the fabrication of chitosan microspheres to create an ideal biomaterial scaffold suitable for transplantation. The surface chemistry of the scaffold can significantly influence cell adhesion and survival while maintaining the “stem-like” properties of cells through integrin receptor signaling pathways. Laminin, fibronectin, gelatin, collagen, and poly-lysine were adsorbed to chitosan to determine their effects on radial cells, which are ventricular zone-derived neural stem cells. Radial cells grown on chitosan adsorbed with fibronectin had the longest processes and their morphology was most reminiscent of primitive radial glia. Radial cells grown on fibronectin tended to have a higher mitotic index (as assessed using Ki67 expression), cell division was high on all substrates. Radial cells, when differentiated on chitosan-fibronectin substrates formed neuronal and glial cell types. Neurons stained positive for TUJ1 and pyramidal markers: FoxP2 (Layer 6), Tle4 (Layer 5,6), Cux1 (Layer 2,3), and Reelin (Layer 1).

Chitosan microspheres were generated by ionic coagulation. Microspheres, optimized to a size appropriate for injection, 25-75 μm were formed using an electrospray technique. Bovine serum albumin (BSA) (1mg/mL) was encapsulated in spheres upon coagulation. Following sphere formation, genipin a natural crosslinker, was added for 4 hours at concentrations of 5.0mM, 0.5mM, or 0.05mM. The higher the molarity and the longer the duration in the genipin fixative, the slower the release rate of the BSA from the spheres. Heparin can also be used to control growth factor release, containing specific binding sites for factors such as FGF and VEGF. Heparin was immobilized to chitosan ionically and covalently using genipin. This result was verified using toluidine blue stain and FTIR. These data show that chitosan microspheres can be fabricated to serve as both a vehicle for delivering drugs and growth factors as well as stem cells for regenerative medicine applications. Supported by grant # 09-3207-BIR-E-2 from the NJ Commission on Brain Injury Research awarded to SWL and CDG.

Mentor: Steven W. Levison(UMDNJ), Chirag Gandhi(NJMS) and Cheul Cho (NJIT)

Nora Ko

Lack of Latent Inhibition in a Rat Model of Anxiety: c-Fos-Related Activation in the Entorhinal Cortex and Cerebellum

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In associative learning, latent inhibition (LI) occurs when pre-exposure to a conditioned stimulus (CS) slows acquisition when that CS is subsequently paired with an unconditional stimulus (US). Reduced LI (comparable learning with and without CS pre-exposures) is observed in schizophrenia and anxiety disorders. Wistar-Kyoto (WKY) rats, a model for anxiety vulnerability, also fail to demonstrate LI, while non-anxious controls (Sprague-Dawley, SD), exhibit the expected delay in acquisition of the conditioned response (CR) during eyeblink conditioning. Simple delay eyeblink conditioning is often associated exclusively with the established cerebellar circuit, but a dissociation has been found in this lab wherein LI of delay conditioning is abolished in SD rats following lesions of the entorhinal cortex (EC), but not the hippocampus. One interpretation of this finding is that pre-exposures reduce CS saliency through alteration of activity in the EC as well as the cerebellum. Accordingly, it was predicted that CS pre-exposure would reduce stimulus-elicited activation in the EC and/or cerebellum of SD but not WKY rats. Conversely, EC and cerebellar activation, and therefore CS saliency, was predicted to be sustained in WKY rats throughout the pre-exposure period. To test this hypothesis, WKY and SD rats were exposed to 0 (context), 1, or 30 auditory CS (82 dB, 500-ms white noise) pre-exposures followed by a delay-type eyeblink conditioning protocol (100 paired CS-US trials with a 30-40 s intertrial interval). Conditioned and pre-exposure-only animals were sacrificed 90 minutes following the onset of the CS exposure, and their brains were processed for c-Fos immuno-reactivity. In conditioned animals, WKYs exhibited deficient LI, replicating previous findings from this lab. SDs exhibited the normal reduced rates of acquisition following pre-exposure. Contrary to expectations, preliminary c-Fos analysis from conditioned animals suggests that WKYs and not SDs show differential activation in the EC, suggesting that our initial hypothesis may need revision. However, this could be due to training effects, and it is expected that the c-Fos analysis from the pre-exposure-only animals will help disambiguate activation due to training, and activation related directly to the CS pre-exposure. Furthermore, the hippocampus, medial septum, and lobule 6 of the cerebellar cortex are additional substrates currently under investigation for potential roles in the modification of incoming signals underlying LI, or lack thereof, in eyeblink conditioning. It is thought that elucidation of such mechanisms in simple learning may translate to humans and thus contribute to the rapidly expanding body of anxiety and psychopathological research.

Mentor: Richard Servatius and Kevin Pang

Pelin Avcu

EFFECTS OF LIPOPOLYSACCHARIDES (LPS) TREATMENT ON CEREBELLAR VOLUME IN AN ANIMAL MODEL OF BEHAVIORAL INHIBITION

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Autism is associated with pathophysiological changes in the cerebellum, particularly an increase volume and decreased numbers of Purkinje cells (PC). The cerebellum comprises an essential neuronal circuitry for the acquisition, expression and retention of conditioned responses (CR) in the delay eyeblink conditioning. Accordingly, faster acquisition of conditioned eyeblink responses is reported in autistics. Toward the development of an animal model of autism, we previously reported that LPS-induced proinflammatory cytokine production during the 2nd trimester of pregnancy induced faster acquisition of eyeblink conditioning in the offspring of Wistar-Kyoto (WKY), but not Sprague-Dawley (SD) rats. Here, we investigate cerebellar neuroanatomy of those male and female SD and WKY rats. Specifically, we determined whether LPS treated WKY rats would exhibit larger cerebellar cortical volumes compared to WKY controls. The granular layer was also included in the analysis since it is the major source of input to PCs and a possible underlying cause of faster acquisition of eyeblink responses. The volume estimation analysis was carried out through the entire left cerebellar cortex of WKY (n=24) and SD (n=26) rats, except for the paraflocculus region to minimize the variability between and within the rats. To analyze the volume, tissue was mounted in gelatin and cut in a coronal plane with a freezing stage microtome. Every 6th section was mounted on gelatin coated slides and then stained with cresyl violet dye. Using computerized assisted stereology, volumes of the tissue sections were estimated using the Cavalieri principle. Granular cell layer-cerebellum ratio was also calculated. Our analysis of tissue revealed that SD rats have significantly larger cerebellar cortex and granular layer volumes compare to WKY rats. Prenatal LPS treatment showed a trend for larger cerebellar cortex and granule cell layer compared to vehicle treatment rats. These data highlight the difficulty in relating structural and functional abnormalities with a direct examination of the interaction of genetic and environmental influences in an animal model of Autism.

Mentor: Richard Servatius

Stacey Cifelli

Downstream targets of mTOR signaling in oligodendrocyte differentiation/maturation

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Our previous study demonstrated that inhibition of mammalian target of rapamycin (mTOR), a downstream target of PI3K/Akt signaling, arrested oligodendrocyte differentiation at the O4+/GalC- late progenitor stage (1). Recent proteomic analysis revealed novel targets and a more complex picture of mTOR function in differentiating oligodendrocyte progenitor cells (OPCs). To further this analysis and search for direct targets of mTOR during oligodendrocyte differentiation we performed iTRAQ-MS analysis of protein samples isolated following 2d and 4d of differentiation in the presence or absence of rapamycin, an mTOR inhibitor. We identified 653 proteins in this analysis, 11% of which were decreased in rapamycin vs. control samples, an additional 11% were upregulated in rapamycin vs. control treated samples. We performed a classification of the changed proteins based on molecular function. We also compared the changes in protein expression to changes in mRNA expression observed by Dugas et al. (2). This allowed for the identification of a subset of changed proteins to be identified as possible translation targets, i.e. changed at the protein level in rapamycin treated samples and either unchanged or increased at the mRNA level at 1-2d of differentiation. We have begun to investigate possible mechanisms of changes in protein expression, in addition to translational control. As the mTOR complexes are serine/threonine kinases, phosphorylation events are an attractive potential mechanism of direct regulation. A possible target for this type of regulation is the transcription factor Yin Yang 1. We have shown changes in post-translational modification of YY1 as well as decreased association of YY1 with HDAC1 with mTOR inhibition during oligodendrocyte differentiation.

1. W. A. Tyler et al., J Neurosci 29, 6367, 2009.
2. J. C. Dugas et al., J Neurosci 26, 10967, 2006.

Mentor: Terri Wood

Swamini Sinha

The role of medial septal/diagonal band GABAergic neurons in proactive interference: effects of selective immunotoxic lesions in latent inhibition.

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The medial septum/diagonal band (MSDB) is a critical structure for learning and memory, yet the functional contributions of its individual neuronal populations (including cholinergic, GABAergic, glutamatergic and peptidergic cells) are still being characterized. Recent studies have implicated a contributing role for the GABAergic MSDB neuronal population, as selective immunotoxic GABAergic lesions of the MSDB (with GAT1-saporin) produce behavioral impairments in spatial and instrumental tasks. Compared to intact controls, rats with GABAergic MSDB lesions are impaired in learning new spatial locations in a delayed match to position procedure and also exhibit a slower rate of extinguishing a previously acquired avoidance response -behaviors that are consistent with an exacerbation of proactive interference. To further establish the role of these neurons in proactive interference, this study examined the effects of selective GABAergic MSDB lesions in latent inhibition (LI) of the classically conditioned eyeblink response. LI in delay eyeblink conditioning is a phenomenon in which pre-exposure to the conditioned stimulus (CS) interferes with the subjects' ability to subsequently associate the CS with an unconditioned stimulus (US), resulting in slower acquisition of the conditioned response (CR). We hypothesized that if damage of GABAergic MSDB neurons increases proactive interference, then rats with selective lesions of these neurons would show facilitated LI. Male Sprague-Dawley rats (n=18) were administered either phosphate-buffered saline or GAT1-saporin via intracranial injection into the MSDB. After 7-10 days of recovery, electrodes were implanted into the upper eyelids of the rats for delivery of US and EMG recording. Conditioning began after another 5-7 days of recovery, with Day 1 consisting of 30 minutes of acclimation to the conditioning context. Day 2 began with either 30 presentations of the CS (82dB, 500ms white noise, 25 -35s ITI) or context pre-exposure of equal duration, followed immediately by 100 paired CS-US trials (82 dB, 500ms white noise co-terminating with a 10V, 10ms square-wave stimulus). In preliminary results, intraseptal GAT1-saporin did not alter CR acquisition in context pre-exposed rats. Rats with GABAergic MSDB lesions continued to exhibit latent inhibition. These preliminary results do not support the idea that damage of GABAergic MSDB neurons increase proactive interference of the classically conditioned eyeblink response. Future studies will examine whether manipulations of the number of CS pre-exposures would facilitate LI in rats with GABAergic MSDB lesions.

Mentor: Kevin Pang

Department of Pharmacology and Physiology

Adetola Shodeinde

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STAT3-mediated apoptosis in cancer cells is independent of STAT1 or STAT2

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Prostate cancer recurs in a refractory form that is non-responsive to current therapies. STAT3 is a transcription factor that was originally discovered as part of the cytokine signaling pathway. A common feature of the hormone-refractory phenotype of recurrent prostate cancer (as well as many other types of cancers) is the dependence on persistently-activated STAT3 for cancer cell survival. Previous studies showed that inhibition of STAT3 activity induced apoptosis in cancerous cells but not benign cells. This suggests that STAT3 is a suitable molecular target for cancer therapy. To mediate its effects on gene expression, phosphorylated homodimers of STAT3 must form and translocate to the nucleus. It has been shown that STAT3 also forms heterodimers with STAT1 and STAT2. Based on these observations, we decided to investigate the role of STAT1 or STAT2 to the survival of STAT3-dependent cancer cell lines. We treated DU-145 and PANC-1 cell lines with STAT1, STAT2, or STAT3 siRNA. Treatment of cancer cell lines with STAT3 siRNA induced apoptosis, while treatment with STAT1 or STAT2 siRNA did not induce apoptosis. When STAT3 siRNA was combined with either STAT1 or STAT2 siRNA in cancer cell lines, percentage of apoptosis did not increase. However, when STAT1-null and STAT2-null fibrosarcoma cell lines were treated with novel STAT3 inhibitors, apoptosis was induced. We also generated a stable cell line of BPH-1 cells expressing a dominant negative form of the STAT3 gene (BPH-DN-STAT3). Treatment with anti-STAT3 oligonucleotides did not induce apoptosis in BPH-DN-STAT3 cells. We can conclude from these results that: (1) neither STAT1 nor STAT2 contribute to the survival of STAT3-dependent cancer cell lines and (2) the novel oligonucleotides designed in our laboratory are mediated primarily through a STAT3-dependent pathway.

Mentor: Beverly Barton

Ammy Santiago

A subpopulation of ventromedial hypothalamic glucose sensing neurons express estrogen receptor alpha (ER α) and are modulated by 17 β -estradiol.

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Females, especially post-menopausal women or those taking high dose oral contraceptives have a higher risk of developing metabolic syndrome, obesity and Type II Diabetes Mellitus. In these women, plasma estrogen levels are severely altered, potentially explaining this increased susceptibility. Estrogen acts as a satiety factor by reducing appetite and increasing energy expenditure, but the underlying mechanisms are still unclear. Estrogen receptor (ER α or ER β) signaling can occur via nuclear- or membrane-associated signaling pathways. In the CNS, membrane-associated estrogen signaling has been shown to alter neuronal membrane excitability and may play a role in energy homeostasis. Glucose sensing neurons within the ventromedial hypothalamus (VMH) are believed to play a critical role in energy and glucose homeostasis. These neurons either increase (glucose-inhibited, GI) or decrease (glucose-excited, GE) their action potential frequency in response to decreased extracellular glucose. The VMH consists of the arcuate (ARC) and the ventromedial hypothalamic nuclei (VMN). Estrogen receptors are widely dispersed within the ARC, but are concentrated within the VL-VMN, a region where we also find a concentration of glucose sensing neurons. Female rodents which lack VL-VMN ER α expression show increased adiposity, decreased energy expenditure, obesity and attenuated sexual receptivity. We hypothesize that estrogen regulates the activity and/or glucose sensitivity of VMH glucose sensing neurons. In order to test our hypothesis, we used whole-cell patch clamp recordings of glucose sensing neurons in combination with immunohistochemistry to determine whether glucose sensing neurons expressed ER α and/or were modulated by estrogen. While a subpopulation of both VL-VMN GE and GI neurons expressed ER α , glucose sensing neurons in the ARC, dorsal- and medial-VMN did not. In addition, in the presence of 2.5mM glucose acute (10min) application of 100nM 17 β -estradiol (17 β E) rapidly depolarized VL-VMN GI neurons and increased their input resistance indicating ion channel closure. These findings suggest that estrogen may acutely modulate VL-VMN glucose sensing neurons in vivo and the absence of estrogen may explain the increased susceptibility for metabolic syndrome in women with altered estrogen levels.

Mentor: Vanessa H Routh

Branly Orban

Effect of Recurrent Hypoglycemia on Acetylcholine-Induced Release of Adrenal Catecholamines

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With the loss of glucose-dependent regulation of glucagon release in type 1 (T1DM) and advanced type 2 diabetes mellitus (AT2DM), epinephrine (Epi) becomes a primary hormone controlling plasma glucose, especially in restoring euglycemia after hypoglycemic episodes induced by pharmacological interventions, such as insulin. However, the Epi response to insulin-induced hypoglycemia (IIH) is progressively blunted in T1DM and AT2DM and in healthy humans after recurrent hypoglycemia (RH). Most investigations of this impairment have focused on glucose sensing in the peripheral and central nervous systems, and on nerve damage due to diabetic neuropathy, while effects of diabetes and RH on the adrenal medulla (AM) have been minimally explored. In studies measuring electrical activity of the adrenal branch of the splanchnic nerve, the change in sympathetic outflow during IIH was similar in control and RH animals, implying that the acetylcholine (ACh)-induced release of adrenal catecholamines may be compromised by RH. To evaluate whether alterations at the level of the AM are involved in the blunted Epi response to IIH, we studied the effects of RH on the ACh-induced release of catecholamines from the adrenal medulla.

Hypoglycemia was induced in male Sprague Dawley (SD) rats with a single subcutaneous (sc) injection of insulin on three consecutive days (RH) while other age-matched rats received sc saline injections. On day 4, RH and some saline-injected animals (AH) were subjected to IIH while measuring plasma catecholamines (Cat), glucagon and corticosterone levels. The remaining saline-injected animals were injected sc with saline on day 4 (Saline). On day 5, left adrenal glands were isolated and perfused to determine Cat release rate evoked by ACh, KCl and selective agonists for muscarinic and nicotinic ACh receptors, pilocarpine (Pilo) and nicotine (Nico), respectively. Cat content was measured in the right adrenal glands.

During day 4 IIH, the time course of glucose decline, minimum plasma glucose levels, norepinephrine and glucagon plasma levels in RH and AH groups were similar. However, the Epi plasma levels at 60 and 90 minutes and the corticosterone levels at 90 and 120 minutes after insulin injection were significantly reduced in the RH group. No differences among RH, AH and Saline animals were found in maximum Cat release rates evoked by ACh (10 μ M to 30mM), ACh concentration producing half-maximal release rates, release rates evoked by 100mM KCl, 1mM Nico, 10mM Pilo. Cat content was unchanged.

Our results show that RH did not change glucose levels during day 4 IIH, though maximal Epi and corticosterone plasma levels were reduced. However, RH did not alter adrenal catecholamine content or release evoked by ACh, Pilo, Nico and KCl. These results indicate that the adrenal responsiveness to ACh is unchanged by RH, and that blunted Epi responses to IIH may result from changes in other mechanisms regulating AM catecholamine release. Since it is likely that many intrinsic regulatory steps in the release of adrenal catecholamines may have been by-passed with our perfusion technique, future studies will evaluate whether adrenal catecholamine release evoked by splanchnic nerve stimulation is affected by RH.

Mentor: Joshua R. Berlin

Chike Cao

Intracellular ATP supports TRPV6 activity via lipid kinases and the generation of PtdIns(4,5)P2

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Transient Receptor Potential Vanilloid 6 (TRPV6) is an epithelial Ca²⁺ channel that plays an important role in Ca²⁺ absorption in the intestines. Activation of these channels has been shown to require phosphatidylinositol 4, 5-bisphosphate [PtdIns(4,5)P₂]. TRPV6 undergoes Ca²⁺-induced inactivation, preventing toxic Ca²⁺ overload of the cell. This inactivation has been shown to be mediated both by Ca²⁺-Calmodulin (CaM) and depletion of PtdIns(4,5)P₂. In addition, cytoplasmic ATP has been proposed to be important for maintaining TRPV6 channel activity in whole-cell patch clamp experiments. To evaluate whether ATP affects channel activity directly or indirectly, we have performed excised inside-out patch clamp measurements with this compound. Channel activity upon excision showed marked current run-down, characteristic of PtdIns(4,5)P₂ dependent channels. Run-down is thought to be due to dephosphorylation of PtdIns(4,5)P₂ by phosphatases present in the patch. We found that ATP reactivated the channels, but only in the presence of Mg²⁺. PtdIns(4,5)P₂ is generated by the sequential phosphorylation of PtdIns into PtdIns(4)P by PI4Ks and the further phosphorylation of PtdIns(4)P by PIP5Ks. This could mean that MgATP provides substrate for lipid kinases present in the patch membrane, allowing resynthesis of PtdIns(4,5)P₂. Three different methods applied in our experiments have verified this hypothesis. First, the effect of MgATP was inhibited by three structurally different compounds LY294002, wortmannin, and PIK93, at concentrations that inhibit type III phosphatidylinositol 4-kinases (PI4Ks). Second, pretreating the patch membrane with a bacterial PI-PLC enzyme significantly inhibited the effect of MgATP. Further, we found that incorporation of the natural long chain PtdIns(4)P into the patch membrane after current rundown inhibited the effect of LY294002 on the MgATP-induced current recovery. PtdIns(4)P had only minimal effect on TRPV6 channel activity. Our data demonstrate that intracellular ATP regulates TRPV6 channel indirectly as a substrate for type III PI4Ks.

Mentor: Tibor Rohacs

Chirag Patel

Investigating possible role of endoplasmic reticulum stress in high fructose induced hepatic steatosis.

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Although it is well established that consumption of high fructose diets results in marked increases in lipogenesis, the mechanism/s underlying this link are not known. Preliminary studies from our laboratory have shown dramatic increases in concentrations of palmitate, oleate and other saturated as well as monounsaturated fatty acids in the liver of rats fed high fructose diets. The potential of palmitate to damage hepatocytes via oxidative stress, endoplasmic reticulum (ER) stress, and dysregulated lipogenesis is well established. In addition, several recent studies have indicated that ER stress contributes to the development of hepatic steatosis. In this preliminary study, we have investigated the potential contributions of (1) ER stress in fructose-induced hepatic steatosis, and (2) dietary palmitate in aggravating the already deleterious effects of dietary fructose on the liver.

Adult rats were fed four different isocaloric diets for 3 months: (glucose + palm oil, glucose + olive oil, fructose + palm oil, fructose + olive oil. Sugars were set at 40% (w/w), lipids at 10% (w/w). At the end of the experiment, accumulation of fat in the liver was evaluated by histology. Biomarkers of ER stress (binding immunoglobulin protein (BiP), spliced X-box binding protein-1 (XBP-1s)) were measured in cytosolic and nuclear fractions of the liver by western blots in as well as by immunocytochemistry. Fructose increased fat accumulation in the liver compared to glucose. Dietary palmitate aggravated hepatic steatosis induced by fructose. Protein expression of BiP and XBP-1s (in whole cell lysate) was higher in the liver of rats fed dietary palmitate. Fructose increased protein expression of XBP-1s in nuclear fractions, independent of type of lipid in the diet. This observation was supported by fluorescent immunohistochemistry which showed greater number of nuclei with XBP-1s in the liver of fructose-fed rats. Although results of this preliminary work showed dietary palmitate to aggravate fructose-induced hepatic steatosis and dietary fructose to increase migration of XBP-1s into the nucleus, further studies are required to establish the role of ER stress in fructose-induced hepatic steatosis.

Mentor: Ronaldo Ferraris

Guoqiang Wang

Homeostasis of Mitochondrial Calcium in Alcoholic Liver Diseases

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Previous studies have demonstrated that alcohol treatment is overtly toxic to hepatocytes; however, the underlying mechanisms by which long-term alcohol consumption progresses to irreversible liver injury are not clear. The prevailing model to explain the pathogenesis of alcohol-induced tissue injury proposes a multifactorial process involving at least two-hits; i.e. the “two-hit” hypothesis. One potential “hit” is the manifestation of oxidative stress during chronic alcohol intake. Ethanol metabolism can directly increase the rates of reactive oxygen species (ROS) production in the mitochondria or at the cytoplasmic face of the endoplasmic reticulum (ER) through the enzymatic activity of cytochrome P450 2E1 (CYP2E1). Excessive alcohol intake can also impair gut barrier function resulting elevated levels of bacterial endotoxins in blood. Endotoxins, such as lipopolysaccharide (LPS), activate Kupffer cells to produce proinflammatory cytokines, such as, tumor necrosis factor α (TNF α) further augmenting ROS production in the liver. Moreover, dysregulation in cytosolic Ca²⁺ homeostasis is predicted to induce mitochondrial Ca²⁺ overload and potentiate the opening of the mitochondrial permeability transition pore (mPTP) leading to mitochondrial damage. Alcohol-induced mitochondrial dysfunction has been proposed to be “second hit” in the pathogenesis of liver disease. Our preliminary studies indicate that chronic alcohol consumption increases total mitochondrial matrix Ca²⁺ levels compared to pair-fed controls. These effects were observed even after overnight culture in the absence of ethanol and in the absence of Ca²⁺-mobilizing hormones or growth factors. We interpret these data to indicate that long-term alcohol consumption may induce adaptive changes in the mitochondrial Ca²⁺ transport. The chronic elevation of mitochondrial matrix Ca²⁺ levels is predicted to stimulate mitochondrial ROS production and contribute to oxidative stress and alcohol-induced tissue injury. In this proposal, we will test the hypothesis that chronic ethanol consumption induces adaptive changes in the mitochondrial Ca²⁺-uptake mechanisms that lead to elevated levels of mitochondrial matrix calcium and enhanced rates of ROS formation.

Mentor: Lawrence D. Gaspers

Hatouf Sukkarieh

Using current-blocking mutants to study Ca²⁺ dependence of Ca²⁺ channel gating

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Voltage-gated calcium channels (CaV1.2 in our study) are highly selective Ca²⁺ permeable channels. This is thought to be due to the presence of multiple Ca²⁺ binding sites in their conduction pathway. The gate in CaV1.2 opens as a result of voltage change sensed by the well conserved positively charged residues located in the transmembrane segments S4 that are away from the ion conducting pathway. It is thought that gating of Ca²⁺ channels is independent from permeation. However, previously published data from our laboratory indicated their linkage. We want to correlate changes in ionic and gating currents of CaV1.2 observed at various extracellular Ca²⁺. Isoleucine (I) substitutions of the highly conserved asparagines (N) in the pore-forming S6 segments of the first and the fourth domains were produced. We also mutated one of the selectivity-filter glutamate (E) to lysine (K) or glutamine (Q) and made double mutants that combine mutations in the selectivity filter and the S6 (N-E to I-K) and (N-E to I-Q). Mutants were transiently expressed in tsA-201 cells. Whole-cell and single-channel currents as well as gating currents (intramembrane charge movements) were recorded in solutions with different extracellular Ca²⁺. Because all mutants produced gating currents, they were functionally targeted to the plasma membrane.

The N428I mutant in the IS6 completely abolished ionic currents carried either by divalent cations, or by monovalent cations at sub- μ M Ca²⁺. The N1499I mutant of the IVS6 abolished currents carried by divalent cations. However, the amplitude of single-channel currents carried by monovalent cations (Na⁺ or Li⁺) at sub- μ M Ca²⁺ was not affected. Unlike in the wild type (WT), gating charge movements in these mutants decreased 2-3 fold upon a change of extracellular Ca²⁺ from 10 to 1mM. Similar change was reported previously to occur in the WT at about 50 μ M Ca²⁺. As expected, the selectivity filter mutants, especially E1145K, decreased Ca²⁺ conductance and increased monovalent currents, which could be readily recorded at 1 mM Ca²⁺ (rather than 1 μ M in the WT). Unexpectedly, the E1145K mutant in the selectivity filter also shifted the Ca²⁺ -dependence of gating charge to the 1mM range. The double mutant E1145K-N1499I passed no inward Ca²⁺ currents at 10 mM Ca²⁺. At the same time, significant outward currents carried by Cs⁺ ions in the intracellular solution could be observed at positive voltages. At 1mM Ca²⁺, the double mutant carried large inward Na⁺ currents at intermediate voltages and large outward Cs⁺ currents at positive voltages. The Ca²⁺-dependence of gating current was as in the E1145 mutant.

Therefore, the N1499I mutation in the channel vestibule specifically diminished gating, or conductance, or both, in Ca²⁺. Currently, we are investigating the combined E1145K-N428I mutant in order to determine if the asparagine in the IS6 is also specifically required for Ca²⁺ conduction and/or gating, or it simply prevents the opening of the gate for any type of cation. Our data reveal that the gating machinery of CaV1.2 channels depends strongly on the type of charge carrier. These findings will impact future drug design and the understanding of multiple diseases linked to mutations in Ca²⁺ channels.

Mentor: Roman Shirokov

Krishna Tobon

D1 dopamine receptor post-transcriptional regulation in cocaine addiction: investigating the role of microRNAs

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A prominent feature of cocaine addiction is behavioral sensitization which is an exaggerated behavioral response to the drug. Both knockout and pharmacological studies have implicated the D1 dopamine receptor as essential in mediating cocaine-induced behavioral sensitization. In this context, molecular mechanisms that regulate expression of D1 dopamine receptor are hypothesized to contribute to cocaine-induced behavioral sensitization. We have determined that the D1 receptor exhibits post-transcriptional regulation (PTR) during mouse brain development and in the mouse CAD catecholaminergic cell line. Our studies using the CAD cell line suggest that the 3' untranslated region (3'UTR) of D1 receptor mRNA is necessary and sufficient for PTR and that it is mediated by microRNAs (miRNAs). The objective of this study was to determine if D1 receptor exhibited PTR in a repeated cocaine administration model, and to determine the mechanisms that mediate D1 receptor PTR. Mice were sensitized using a binge administration paradigm in which saline or cocaine (15mg/kg) was administered 3 times per day, 1 hour apart, for seven days. The sensitized animals exhibited both locomotor sensitization and D1 receptor PTR in the striatum, wherein we observed an increase in D1 receptor mRNA with no concomitant increase in D1 receptor protein levels. A cocaine challenge dose administered to cocaine-sensitized animals rapidly increased D1 receptor protein expression in a brain region-specific manner. Global genome expression profiling identified several miRNAs whose expressions were specifically altered in cocaine-sensitized mice. To identify the miRNAs regulating D1 receptor PTR in cocaine-sensitized mice, candidate miRNAs were selected based on two criteria: those that (1) showed significant increase in expression, and (2) had putative binding sites in the D1 receptor 3'UTR. The ability of candidate miRNAs to mediate D1 receptor PTR was functionally tested in the CAD cell line using reporter constructs with 3'UTR from the D1 receptor gene. The results suggest that regulation of D1 receptor expression by miRNAs might contribute to cocaine-induced behavioral sensitization.

Funded by grants from NIH (DA026030 & DA026030-02S1) to EVK and a PhRMA Foundation Pre-Doctoral fellowship and Alfred P. Sloan Minority Foundation fellowship to KT.

Mentor: Eldo V. Kuzhikandathil

Lihong Hao

The Role of NPY-GI Neurons in Disease Related Anorexia-Cachexia Syndrome

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The arcuate nucleus (ARC) within the ventromedial hypothalamus (VMH) contains NPY neurons which are involved in regulation of food intake and energy balance. Cell imaging and patch clamp studies show that ~40% of ARC NPY neurons are glucose inhibited (GI) neurons. GI neurons depolarize and increase their input resistance in response to decreased glucose levels. This effect is mediated by AMP-activated protein kinase (AMPK) activation. Previous studies in our lab and others show that fasting increases *cfos* expression in NPY neurons. Moreover, we showed that fasting enhances hypothalamic NPY release, phosphorylation of VMH AMPK and depolarization of NPY-GI neurons in response to decreased glucose. During anorexia-cachexia, the neuronal regulation of food intake is interrupted and the brain no longer responds to energy deficit. Lipopolysaccharide (LPS) injection, an animal model of disease related anorexia-cachexia, blocks fasting induced *cfos* expression in NPY neurons. We hypothesize that LPS will also inhibit the increased AMPK phosphorylation and excitation of NPY-GI neurons in response to decreased glucose following fasting. Furthermore, the effects of LPS will be mediated via inflammatory cytokines. To test this, we used saline or LPS (40 $\mu\text{g}/\text{mouse}$) injected 4-6 week old C57Bl/6 mice which had been fasted for 24 hours. LPS inhibited fasting stimulated food intake post-refeeding and decreased body weight. LPS also blocked the fasting-induced increase in VMH pAMPK α 2 in response to decreased glucose from 2.5 to 0.1 mM ($P < 0.05$; $N = 8/\text{group}$; control: fed with saline injected). Next, VMH brain slices were exposed to decreased glucose in the presence and absence of the inflammatory cytokines tumor necrosis factor alpha (TNF α ; 40 ng/ml) or interleukin 1-beta (IL-1 β ; 10 ng/ml). Both TNF α ($P < 0.05$; $N = 5/\text{group}$) and IL-1 β ($P < 0.05$; $N = 5/\text{group}$) blocked the increased VMH pAMPK α 2 in response to decreased glucose. Whole cell current clamp recordings of GI neurons from NPY-GFP and wildtype mice were used in order to determine whether TNF α blocked the fasting-induced changes in the glucose sensitivity which we observed previously. The percent change in IR of NPY-GI and GI neurons from 2.5 mM to 0.1 mM glucose in the presence and absence TNF α was used to quantify changes in glucose sensitivity. Preliminary results suggest that TNF α blunted the increase in input resistance normally seen with a glucose decrease from 2.5 mM to 0.1 mM. These data suggest that LPS and inflammatory cytokines block the stimulatory effect of fasting on the phosphorylation of AMPK and excitation of GI neurons in response to glucose decreases. This may contribute to disease-related anorexia-cachexia.

Supported in part by NIH CA R21 139063.

Mentor: Vanessa Routh

Paula Green

The Role of hTERT (human telomerase reverse transcriptase) in modulating autophagy

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Telomerase is a reverse transcriptase composed minimally by a template RNA (in humans hTR) and a protein subunit that mediates catalysis (TERT). Together they work in concert to maintain telomeres. Emerging evidence has indicated that hTERT is also present in mitochondria particularly under oxidative stress, where it accumulates in the organelle. Contradictory reports indicate that hTERT in mitochondria either protects or sensitizes the cells to oxidative stress-mediated mitochondrial DNA (mtDNA) damage and cell death. Here we used various approaches to demonstrate that hTERT does not affect antioxidant defenses or the cells ability to remove oxidative mtDNA damage. We also show that hTERT binds mtDNA but under oxidative stress such interaction is lost. Finally, we demonstrate the ability of hTERT to modulate oxidative stress-induced autophagy, which is associated to its subcellular localization. Our results reveal a crosstalk between telomerase and autophagy, unveiling new details about how telomerase impacts the cellular response to oxidative stress.

Mentor: Janine H. Santos

Reema Patel

Proinflammatory Cytokines Inhibit Ventromedial Hypothalamus (VMH) Glucose Sensing

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Obesity is a major risk factor for type 2 diabetes mellitus. The two disorders often present concurrently and are accompanied by hyperleptinemia and hyperinsulinemia. Interestingly, proinflammatory cytokines such as tumor necrosis factor- α (TNF α) and interleukin-1 β (IL1 β) are also elevated. This observation has led to the hypothesis that obesity is an inflammatory disease. While the functions of insulin and leptin in energy homeostasis have been studied extensively, less is known about the roles of proinflammatory cytokines. The ventromedial hypothalamus (VMH) plays a key role in the regulation of energy homeostasis and is a site of leptin and insulin action. Proinflammatory cytokines may also act in the VMH. The VMH contains glucose inhibited (GI) neurons that are believed to play an important role in energy balance. GI neurons increase their electrical activity as extracellular glucose levels decrease. The mechanism by which this occurs involves the metabolism of glucose which increases the ATP:AMP ratio. Glucose deficit increases AMP, resulting in the phosphorylation and activation of AMP-activated kinase (AMPK). AMPK closes a chloride channel, resulting in depolarization and increased action potential frequency. We have previously shown that leptin inhibits AMPK and blunts the response of VMH GI neurons to decreased glucose. Since leptin is also classified as a cytokine, we hypothesize that proinflammatory cytokines act similarly. In support of this, we found that a proinflammatory cytokine cocktail (40ng/mL TNF α and 10ng/mL IL1 β) inhibits VMH AMPK phosphorylation in response to decreased glucose from 2.5 to 0.3mM (40% decrease in AMPK Thr172 phosphorylation; $p < 0.05$) in adult mice. Furthermore, using membrane potential dye imaging, we have found that IL1 β (10ng/mL) inhibits the response of VMH GI neurons to decreased glucose from 2.5 to 0.7mM (% cells responding=9.8% control vs 6.1% IL1 β ; $p < 0.05$). These data suggest that proinflammatory cytokines may play a role in energy homeostasis by regulating the glucose sensitivity of VMH GI neurons.

Mentor: Vanessa H Routh

Ruifang Zheng

Nitric oxide inhibition of a low affinity cationic amino acid transporter

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The cationic amino acid transporter 2A (CAT2A) is a low affinity, high capacity amino acid carrier expressed in cardiomyocytes and other tissues. Previous studies in our lab showed that nitric oxide (NO) is able to modulate its own biosynthesis through the non competitive inhibition of CAT2A transport activity. Since this inhibition appears to be the result of a direct interaction between NO and CAT2A, we propose that NO modulates CAT2A activity through S-nitrosation. S-nitrosation is the chemical reaction by which cysteine residues of proteins are modified by NO. To test this hypothesis, first, we added a FLAG tag on the N-terminus of mouse CAT2A (mCAT2A) and over expressed it in COS-7 cells. COS-7 cells expressing mCAT2A exhibit significant L-lysine uptake activity which can be inhibited by 100 μ M of the NO donor SNAP (S-Nitroso-N-Acetyl-D,L-Penicillamine). Western blot and biotin switch assays demonstrated that 100 μ M of another NO donor, GSNO (S-nitrosoglutathione), can cause S-nitrosation of mCAT2A. We generated cysteine to alanine site-directed single point mutations within mCAT2A to determine which cysteine residues are involved in NO modulation. We chose Cys347, 427, and 171 as initial targets because of their putative location in transmembrane segments near or within cationic amino acid binding sites. The Cys347Ala mutant retains approximately 35 % of transport activity compared to the wild type. However, a 7-min long incubation with 100 μ M SNAP fails to inhibit its transport activity. Western blots and immunofluorescent staining demonstrate that the membrane expression levels of Cys347Ala mutant do not decrease compared to wild type. The Cys427Ala mutant shows higher transport activity than the wild type and 100 μ M SNAP inhibits about 50% of total uptake. Cys171Ala mutant has similar transport activity as the wild type, and SNAP completely inhibits its uptake activity. Based on the above data, we conclude that: 1) Addition of a FLAG to mCAT2A has no effect on uptake levels. 2) Cysteine 347 is important for NO modulation of mCAT2A as well as its basal level transport activity. The decreased activity of the Cys347Ala mutant is not due to decreased membrane expression levels. 3) The cysteine 427 residue partially contributes to the sensitivity to NO inhibition. 4) Cysteine 171 is not involved in NO modulation of mCAT2A activity. 5) NO inhibition of CAT2A could occur through S-nitrosation of cysteine residues 347 and 427. Nonetheless, there are 15 cysteines in mCAT2A and we are working on other Cys mutants to determine their roles in NO modulation of this transporter.

Mentor: Daniel Peluffo

Samantha Cote

Characterization of D3 Dopamine Receptor Signaling in a Parkinson's Disease Mouse Model

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The dopamine D3 receptor is known to couple to many signal transduction pathways and has been implicated as an important player in Parkinson's disease (PD). When the D3 receptor undergoes chronic agonist application, the D3 receptor exhibits a tolerance property where repeated stimulation induces a decreased response without receptor internalization. In vitro studies have shown that the D3 receptor tolerance extends to other downstream D3 coupled signal transduction pathways such as the P/Q type Ca⁺ channels, GIRK channels and phosphorylation of Mitogen Activated Protein Kinase (MAPK). It is believed tolerance plays a role in Parkinson's Disease, especially in those patients chronically taking the drug Levodopa (L-dopa), which is a dopamine replacement therapy. These patients, after chronic L-dopa treatment, develop secondary dyskinesia as a side effect of the drug termed Levodopa Induced Dyskinesia (LID). To test whether the D3 tolerance property exists in an in vivo PD animal model, we have used an established PD mouse model using the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a novel Ddr3-EGFP mouse for accurate identification of the D3 receptor in the mouse striatum, the brain region depleted of dopamine. Normal mice were also chronically treated with L-Dopa as well as a subset of the MPTP treated mice and differences in MAPK phosphorylation were measured via western blot. MPTP treated mice exhibited an increased MAPK phosphorylation when challenged with a D3 selective agonist, PD128907. However, the MPTP group chronically treated with L-dopa showed a decrease in phospho-MAPK after administration of PD128907 when compared to those mice given saline. Taken together, these results suggest that changes in D3 and downstream MAPK phosphorylation occur in PD-LID mouse model.

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Mentor: Eldo Kuzhikandathil

Thomas Comollo

Molecular Modeling and Structure – Function Analysis of Calcium Channel Gamma Subunits

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Voltage gated calcium channels are found in many types of neurons, muscle cells, and other types of excitable cells. These channels are comprised almost always of an alpha and a beta subunit and sometimes an alpha2delta and/or a gamma subunit. The example of voltage gated calcium channels being examined in our study are CAV1.2 channels. These are L-type (for “long” inactivation) channels, which are activated at relatively high voltage activated channel. We are interested in the difference of the interaction of calcium channel gamma subunits 1 and 6 with these channels. Currently, gamma 1 is known to increase the likelihood of inactivation, causing inactivation of CAV1.2 channels at more negative voltages. gamma 6 alternately is known to have smaller, if any, effects on inactivation. Unlike gamma 1, gamma 6 decreases current density flowing into cells transfected with CAV1.2 channels. These differences exist while the two gammas share 38% sequence identity and 68.5% homology.

In order to determine the structure – functional differences between subunits gamma 1 and gamma 6, we computationally folded components of these two proteins and have “stitched” those components together into models. Subsequently, we have run these models in molecular dynamics simulations to arrive at several “live” models of these calcium channel subunits. These structures will be fundamental in explaining the functional effects of mutations on functionally important features of the molecules. This includes the partially ordered, intracellular N-terminal “helix sandwich” feature of gamma 6, not present in gamma 1. This also includes the N-linked glycosylation found in and bent-helix, triple beta sheet structure of the first extracellular loop. This loop has been reported in the literature and observed in our own mutants as important for the gamma’s effects on L-type channels.

Mentor: Roman Shirokov

Viktor Lukacs

Phosphoinositide regulation of TRPV1 channels

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TRPV1 is a nonselective calcium permeable cation channel present in polymodal nociceptors that plays a crucial role in the development of inflammatory pain and thermal hypersensitivity. Plasmamembrane phosphoinositides are recognized as important regulators of TRPV1; the precise nature of their effect is, however, controversial. We and others have shown that phosphatidyl inositol (4,5) biphosphate [PI(4,5)P₂] as well as other phosphoinositides activate TRPV1 in excised patches. Calcium influx via TRPV1 channels activates PLC and results in a robust depletion of both phosphatidyl inositol (4) phosphate [PI(4)P] and PI(4,5)P₂ in expression systems. Hydrolysis of PI(4,5)P₂ under these circumstances is now accepted to be an important contributor to channel desensitization. However, the involvement of PI(4)P in the process remains an issue of debate. In addition, preceding data corroborated by our own observations suggest an additional indirect inhibitory effect of PI(4,5)P₂, but not other phosphoinositides. In the present work we show that potentiation of whole-cell TRPV1 currents by bradykinin is partially impaired by dialyzing diC8-PI(4,5)P₂ through the patch pipette in both expression systems and native cells (DRG neurons), supporting the notion that in addition to the well documented role of PKC-mediated phosphorylation in TRPV1 channel sensitization, PI(4,5)P₂ depletion may also be involved in this phenomenon. Using mouse DRG neurons we confirm that in addition to PI(4,5)P₂, PI(4)P is also an important activator of TRPV1. Both lipids are simultaneously depleted in response to TRPV1-activation, while dialysis of both lipids via the patch pipette reduced desensitization of TRPV1-positive neurons. In contrast Bradykinin receptor activation differentially regulated PI(4,5)P₂ and PI(4)P abundance in DRG neurons, resulting in isolated PI(4,5)P₂ depletion. These observations suggest important differences in phosphoinositides handling during calcium-activated and receptor-induced PLC activation, respectively, and may partially explain the differential TRPV1 regulation under these conditions.

Mentor: Tibor Rohacs

Vishwendra Patel

Role of muscle specific kinase (MuSK) in motor nerve function in mouse model of MuSK Myasthenia Gravis.

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Myasthenia gravis (MG) is a disorder of the neuromuscular junction (NMJ) due to circulating auto-antibodies which silence endplate acetylcholine receptors (AChR) to cause muscle weakness. Muscle specific tyrosine kinase (MuSK) is central to the cascade of muscle proteins that interact to form and maintain the NMJ. Myasthenia gravis associated with auto-antibodies to MuSK (MuSK-MG) was first described in 2001 (Hoch W. et al. Nat Med 7: 365-368). MuSK antibodies are presumed to disrupt AChR clustering on the muscle endplate surface. MuSK-MG patients are resistant to acetylcholinesterase (AChE) inhibition and are less responsive to corticosteroids, as compared to AChR-MG patients. To help improve understanding and treatment of MuSK-MG, we study a mouse model of MuSK-MG. To explore the role of MuSK in presynaptic function we also propose to study motor nerve function in shRNA mediated MuSK knock down mice. We sub cloned the ectodomain from the complete rat MuSK cDNA. Rat MuSK ectodomain fragment was then inserted in pGEpi vector and expressed in E. Coli. Rat MuSK ectodomain was then purified on a Ni/Cd column followed by an ion exchange column. We immunized 8 weeks old male B6 mice with 10 µg of purified rat MuSK ectodomain. Mice were immunized by three successive (at 4 week intervals) subcutaneous injections of 10 µg of purified rat MuSK ectodomain emulsified in 100 µl of equal volumes of PBS and complete Freund's adjuvant (CFA) containing M.tuberculosis, strain H37Ra in the flank region. Control mice received only PBS+CFA mixture. We collected the sera from the immunized mice to check for presence of MuSK antibodies. To evaluate the effects of MuSK-MG on muscle strength and co-ordination, we measured the time mice could walk on a rod rotating at 20 RPM. To study the effect on respiratory functions we performed head-out whole body plethysmography on MuSK-immunized and on control mice. In addition, isolated hemi-diaphragm muscles were stained with fluorescent bungarotoxin in order to detect MuSK antibody associated alterations of AChR clustering at the endplates. Finally, conventional microelectrode techniques were used to record spontaneous and stimulus evoked endplate potentials in order to evaluate the safety factor of synaptic transmission. Our results demonstrate that within two weeks after the second injection of MuSK ectodomain, mice showed significant weakness and their time to walk on the rotarod decreased compared to vehicle alone treated controls. Treated mice also showed significant changes in their respiratory functions. The amplitude of endplate potentials and miniature endplate potentials were also reduced in MuSK immunized mice compared to the controls. Two of the MuSK MG mice showed failures of neuromuscular transmission in response to high frequency nerve stimulation. These findings support the hypothesis that study of alterations at both the presynaptic and postsynaptic sides of NMJ in MuSK-MG can explain the unique clinical features of MuSK+ MG and reveal precise targets for therapeutic intervention.

Mentor: J.J. McArdle

WEIWEI WANG

Mechanism for Photoreceptor Axon Retraction after Retinal Detachment

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Photoreceptors, including cones and rods, are the sensory neurons of the vertebrate retina. They are photosensitive and capable of converting photon signals into a biological response. In response to injury or diseases, photoreceptor cells display synaptic plasticity. Thus the connection between photoreceptor cells and secondary neurons in retina can be affected. We are trying to understand the mechanisms of rod cell axon retraction which occurs after retinal detachment and in some retinal diseases. Retraction can disconnect the first synapse in the visual system and thereby lead to the loss of visual function.

RhoA has been reported to be involved in the retraction of growth cones during development and to inhibit new growth after injury. Previous studies in our lab demonstrated that inhibition of RhoA and its downstream effector Rho kinase (ROCK) blocks rod cell axon retraction. Here we show that inhibiting a downstream effector of ROCK, LIM kinase (LIMK), also blocks retraction. Our data demonstrate significant difference between control group and LIMK inhibitor (LIMKi) treated group: 35% decrease of axon length of rod cells in control group after 7 hours incubation, compared with axon length 1 hour after dissociation; whereas only a 15% decrease was found in LIMK inhibitor treated group.

Furthermore, treating retinal explants with Nicardipine (Nc) also blocked retraction in previous research in our lab. This might be attributed to the reduced myosin light chain (MLC) contractile force, which can be regulated by Ca²⁺/calmodulin activity. In our study here, we treated dissociated rod cells with Nc plus LIMKi. Preliminary data showed that morphology of 40% of the rod cell axon terminals remained static after three days culture in treated groups compared with control group, in which axon terminals either retract completely or lose their structure. LIMKi treatment alone resulted in less than 10% of rod cells axon terminal remaining static, however, none of such axon terminals was found in control group. These results suggest that LIMK and Calcium/calmodulin target different downstream effectors to regulate axon retraction.

We hypothesize that retinal detachment, which produces a spreading depression, opens Ca channels and triggers actomyosin contraction; to explain RhoA activity, we think ATP, which is also produced during spreading depression, might activate RhoA through binding to purinergic receptors. In the future, we will continue to study the signaling pathway in photoreceptor axon retraction to explore for therapeutic targets.

Mentor: Ellen Townes-Anderson

Xiaoting Hong

Gap Junctions Propagate Opposite Effects in Normal and Tumor Testicular Cells in Response to Cisplatin

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Therapeutically beneficial toxic effects can be propagated among tumor cells through gap junctions during chemotherapy and radiotherapy. However, toxic intercellular signaling has the potential to enhance killing of normal cells by the same mechanism. We investigated whether the predominant effect of gap junctional intercellular communication (GJIC) on cell survival following 1 h exposure to a toxic chemotherapeutic agent (cisplatin) differed between normal and tumor testicular cells. As expected, cisplatin-induced killing was enhanced when testicular tumor cells were cultured at high density (with GJIC) relative to low density (without GJIC). In contrast, cisplatin-induced killing of normal testicular cells was dramatically decreased in high density culture. The opposite effects of high density culture on cisplatin-induced cell death in the normal and tumor cells were found to be entirely due to the presence of functional gap junctions. Block of GJIC in high density cultures by chemical inhibition or by siRNA increased cisplatin cytotoxicity in normal cells and decreased it in tumor cells. The decreased toxicity due to GJIC in normal cells correlated with reduction of DNA interstrand crosslinks (ICLs), but in tumor cells ICL formation was unaffected. Thus, with equal toxic exposure to a chemotherapeutic agent, GJIC communicated predominantly protective signals among the normal cells, reducing overall toxicity, whereas in tumor cells, GJIC communicated predominantly toxic signals, increasing overall toxicity. These results suggest that even short-term upregulation of GJIC may be a profitable strategy for cancer therapy that simultaneously sensitizes tumor cells and decreases off-target toxicity, enhancing overall therapeutic profile.

Mentor: Andrew L Harris

Microbiology and Molecular Genetics

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ORF7 of Varicella Zoster Virus is a Neurotropic Factor

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Varicella zoster virus (VZV) is the causative agent of chickenpox and herpes zoster (shingles). After the primary infection, the virus remains latent in sensory ganglia, and reactivates upon weakening of the immune system due to various conditions, erupting from sensory neurons and infecting surrounding skin tissue. The factors involved in neuronal invasion and establishment of latency are still elusive. Screening of non-essential VZV gene deletion mutants in differentiated neurons led to identification of open reading frame 7 (ORF7) as a neurotropic factor. ORF7 deletion caused loss of polykaryon formation in vitro and severely impaired viral spread in human skin and nervous tissue. Based on these observations, ORF7-deleted VZV is proposed as a safe neuroattenuated vaccine candidate against chickenpox and shingles.

Mentor: Hua Zhu

Atul Khataoakar

Understanding cell cycle control in *Caulobacter crescentus*

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Caulobacter crescentus is a Gram-negative α -proteobacteria found in fresh water. Planktonic *C. crescentus* are motile swarmer cells, which divide asymmetrically to form motile and non-motile daughter cells. Non-motile daughter cells express a stalk that enables them to attach to surfaces while the swarmer cells encode a flagellum and pili. The swarmer and stalk cells are morphologically distinct and are genetically programmed to follow different cell cycle patterns.

Swarmer cells are unable to initiate DNA replication immediately after cell division. They must first differentiate into stalked cells before DNA replication can initiate. During this cellular transformation, they replace their flagellum and pili with a stalk. Unlike swarmer cells, stalked cells can initiate DNA replication immediately after cell division giving rise to another stalked cell and a swarmer cell. This asymmetric division of *C. crescentus* is a result of tightly controlled DNA replication, which occurs only once every cell cycle.

The response regulator protein CtrA controls the *C. crescentus* cell cycle. CtrA is a transcription factor that binds to the chromosomal origin of replication, inhibiting initiation of replication. In addition to its regulation by phosphorylation and proteolysis, CtrA's activity is tightly controlled by a small protein called SciP. SciP binds to CtrA and inhibits its interaction with RNA polymerase. SciP is conserved in all bacteria containing CtrA homologs, and how SciP and its homologs bind to and regulate their target response regulator proteins is unknown.

Our goal is to determine how SciP functions mechanistically to inhibit CtrA function in *C. crescentus*. To accomplish this goal we are using biochemical, genetic, and X-ray crystallographic approaches to study the SciP-CtrA interaction. In addition to revealing the mechanistic basis of response regulator inhibition by SciP family proteins, the information gained from my structure-function analysis of CtrA-SciP may guide the development of drugs that function to inhibit bacterial response regulator proteins by mimicking the effects of SciP.

Mentor: Matthew Neiditch

Fabiana Di Sanzo

An Investigation of viral genomic packaging in the Cystoviridae, a family of segmented dsRNA bacteriophage

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One of the least understood processes in the replication of a dsRNA virus is its mechanism of genomic packaging. In bacteriophage phi6, the best characterized member of the Cystoviridae, it has been shown that its segmented dsRNA genome is acquired by filling an already assembled particle, using a mechanism involving recognition and translocation of plus-strand transcripts into a preformed dodecahedral core. The packaging model describes packaging to occur serially, in the segment order Small, Middle, then Large, and is dependent on segment specific packaging signals. In phi6, these pac signals are about 200nt at the 5' end of the plus-strand transcripts, and demonstrate extensive secondary structure. Although critical for segment selection, the mechanism in which these signals are recognized by the procapsid still remains in question. Based on previous studies, P1, the major structural protein, is the major candidate for segment binding. This investigation focuses on genomic packaging in ?2954, a recent member of the Cystoviridae, in order to better understand how the segments are selected by the procapsid. Mutational analysis of the pac sequences and reverse genetics delineate the extent and stringency of the pac signals and help identify segment binding sites on the procapsid. This investigation demonstrates that ?2954 may not be as stringent as other members of the Cystoviridae, suggesting variation across the family. Also, packaging experiments using phage that were mutagenized in nitrosoguanidine, resulted in the isolation of phage which allow WT-like acquisition of S and M segments harboring pac mutations that normally reduce packaging ten-thousand fold. Analysis of these phage revealed an RNA suppressor mutation in the M segment pac signal that rescues packaging to WT. Furthermore, experiments exchanging pac signals between segments confirm a serially-dependent packaging model in which the segments are packed in the order S, M, then L. Interestingly, the Cystoviridae are structurally similar to the Reoviridae, having segmented dsRNA genomes, and similar cores. It is unknown how the Reoviridae precisely package their segments, but since they are structurally similar to the Cystoviridae, they may share a similar mechanism as described in this investigation.

Mentor: Leonard Mindich

Iлона Tala

A p210 BCR/ABL Mutant That Lacks Guanine Nucleotide Exchange Factor Activity Induces Erythroleukemia in a Murine Bone Marrow Transplantation Model

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The p210 BCR/ABL and p190 BCR/ABL fusion proteins are constitutively active tyrosine kinases that are associated with chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL) respectively. Whereas both of these fusion proteins contain equivalent contributions from ABL, they differ in the amount of BCR sequence at the NH₂-terminus. We have identified a domain with guanine nucleotide exchange factor (GEF) activity which is contained within the BCR sequences of p210 BCR/ABL that distinguishes it from p190 BCR/ABL. This activity is subject to autoinhibition in the context of BCR, but is constitutively active in p210 BCR/ABL, and cannot be blocked using the tyrosine-kinase inhibitor, Imatinib mesylate. The activities of small GTPases are regulated by GEFs, and are essential for the regulation of hematopoietic stem cell differentiation and proliferation. In a human Ph-positive erythroleukemia cell line (K562), inhibiting p210 BCR/ABL-mediated Ras activation induces erythroid differentiation, while abnormal activation of Cdc42 reduces erythroid progenitors and erythroid colony-forming units in bone marrow. In order to directly investigate the contribution of the GEF activity to p210 BCR/ABL-mediated leukemogenesis, we have introduced a point mutation into p210 BCR/ABL (p210 BCR/ABL(S509A)) that eliminates the activity. The mutation has no discernable effect on tyrosine kinase activity, which is consistent with previous data that the two activities are functionally independent. We have compared p190 BCR/ABL, p210 BCR/ABL, and p210 BCR/ABL(S509A) in a murine bone marrow transplantation (BMT) model. Although all transplanted mice develop splenomegaly with leukocytosis, the p190 BCR/ABL and p210 BCR/ABL(S509A) transplanted mice exhibit a more rapid onset of disease than mice transplanted with p210 BCR/ABL (15-18 days vs 23-38 days). This difference is observed regardless of whether the donor mice are pre-treated with 5-Fluorouracil. The more rapid onset of disease induced by p190 BCR/ABL and p210 BCR/ABL(S509A) is associated with reticulocytosis, and increased numbers of nucleated red blood cells (nRBC), but no anemia. The nRBC/100 WBC counts are 0, 4.75 ± 2.21 , 277.5 ± 190.6 , and 119.5 ± 66.0 for MIG, p210 BCR/ABL, p190 BCR/ABL and p210 BCR/ABL(S509A) respectively, thus suggesting leukemic expansion of the erythroid lineage. To confirm this difference in lineage expansion, clonogenicity assays were performed. Consistent with previous reports, p210 BCR/ABL supports the growth of CFU G/M, but not BFU-E, and colony growth is completely inhibited by Nilotinib. In contrast, p190 BCR/ABL and p210 BCR/ABL(S509A) support the growth of BFU-E (but not CFU G/M), and these colonies are completely insensitive to Nilotinib treatment. In summary, our observations suggest that a point mutation that eliminates the GEF activity of p210 BCR/ABL produces a phenocopy of p190 BCR/ABL, without affecting the tyrosine kinase activity. These results support a model in which the GEF activity that distinguishes p210 BCR/ABL from p190 BCR/ABL actively regulates disease progression by determining lineage-specific leukemic expansion.

Mentor: Ian Whitehead

Jessica Kaplunov

Telomere Dysfunction Induced Cellular Senescence in Human Breast Cancer Precursor Lesions

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Cellular senescence is a stable proliferative arrest that can function as a potent tumor suppressing mechanism in several mouse model systems. Senescent cells have been detected in benign precursor lesions to human cancers, suggesting that this permanent growth arrest limits cancer progression at pre-malignant stages in humans. Here we test a potential role for cellular senescence in suppressing the growth of breast cancer.

Cultured human mammary epithelial cells (HMECs) encounter two barriers to cellular proliferation. The first barrier, called stasis, is triggered in response to cell-extrinsic stresses such as inadequate culture conditions and is dependent on p16INK4a/Rb signaling. The second barrier is p53 dependent and has previously been termed agonescence. We demonstrate that agonescence is a response to dysfunctional telomeres and thus term it telomere dysfunction induced senescence (TDIS). In contrast, cells in stasis did not display dysfunctional telomeres demonstrating that stasis occurs independent of telomere dysfunction. Both, cells in stasis and TDIS, however, displayed high levels of proteins involved in senescence associated heterochromatin formation, including macroH2A. Similarly, telomere dysfunction induced by overexpression of dominant defective TRF2 mutant also resulted in HMEC senescence and upregulation of macroH2A. Analysis of pre-malignant and malignant human breast cancers, revealed a massive and homogeneous accumulation of senescent cells displaying elevated macroH2A levels in early lesions, such as ductal hyperplasias, but not in ductal carcinomas in situ (DCIS) or in malignant breast carcinomas. Our data therefore suggest that cellular senescence limits breast cancer progression at stages prior to DCIS. Senescent cells generally lacked p16 expression but consistently displayed multiple dysfunctional telomeres and elevated levels of macroH2A, and mirrored the senescence phenotype of senescent post-stasis cell cultures. Our data demonstrate that TDIS is an in vivo physiological response of HMECs in hyperplastic regions and suggest that TDIS suppresses breast cancer progression at pre-malignant stages in humans.

Mentor: Utz Herbig

Jessica Mann

Characterization of ComGG, minor pseudopilin of *Bacillus subtilis*

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ComGD, ComGE, and ComGG, termed the minor pseudopilins, are all essential for maximal DNA-binding and uptake during natural transformation in *B. subtilis*. The minor pseudopilins of the *Escherichia coli* type-2 secretion system (T2SS) form a complex, as do the minor pilins of T4 pili. Little is known about the interactions of the *B. subtilis* minor pseudopilins. ComGG is encoded by the last gene in the comG operon and is therefore the most accessible for genetic manipulation. I have mutagenized comGG and characterized the resulting transformation deficient mutants. A few of these mutants have been subjected to nitrosoguanidine-induced mutagenesis, in order to produce suppressor mutations. Mapping of the suppressor mutations will identify proteins that work in conjunction with ComGG. Additionally, with the use of ComGG antisera, we have completed a large-scale ComGG co-immunoprecipitation from the membranes of competent *Bacillus*. Using mass spectrometry, we hope to further reveal the binding partners of ComGG.

Mentor: David Dubnau

John Mavrianos

Identification and Characterization of a Novel Two-Component Response Regulator in *Candida albicans*

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Two-component signal transduction systems allow fungi to sense and respond to a variety of environmental stimuli. We report here identification and characterization of a previously uncharacterized (orf19.583) two-component response regulator gene from *Candida albicans*. Because of its apparent functions in stress (oxidative and osmotic) adaptation, we have named this gene SRR1 (stress response regulator). SRR1 appears to be unique to the *Candida* clade characterized by the unique translation of CUG codons as serine rather than leucine. The SRR1 gene has an open reading frame of 849 bp which encodes a 282 amino acid protein with an estimated molecular mass of 32 Kd. The response regulator domain present at the C-terminus (154-270 aa) of this protein has the characteristics of a prototypical response regulator, including the conserved aspartate (putative site of phosphorylation) and lysine residues. The gene deletion mutants lacking SRR1 were more sensitive to both osmotic and oxidative stress compared to the wild-type and gene reconstituted strains. Furthermore, the null mutant exhibited a filamentation defect on hyphae-inducing growth media. Virulence studies also show that SRR1 is essential for the pathogenesis of *C. albicans* in a mouse model of disseminated candidiasis. Finally, a point mutation at the putative site of phosphorylation led to a phenotype similar to that of the null mutant, further strengthening the notion that SRR1 is indeed a response regulator. In conclusion, this study shows for the first time that *C. albicans* possess an additional response regulator gene and that at least in the case of the two-component signaling in *C. albicans*, the functional circuitry has assumed a more expansive role than previously reported.

Mentor: Neeraj Chauhan

Jonathan Guito

THE CELLULAR PEPTIDYL-PROLYL CIS/TRANS ISOMERASE PIN1 REGULATES REACTIVATION OF KSHV FROM LATENCY

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). We and others have demonstrated that the KSHV Rta protein functions as the lytic switch to reactivate latent virus. Rta forms ternary complexes with the Notch pathway effector RBP-JK and promoter DNA to specify genes for transactivation. Although Rta expression is necessary for initiating KSHV reactivation, our data suggest that Rta acts inefficiently as the lytic switch. We hypothesize that Rta's inefficiency plays a central role in the balance between expression of viral lytic cycle oncogenes and lysis of the host cell.

We have previously demonstrated that Rta tetramers, but not higher-order multimers, are required for Rta-mediated transactivation and reactivation. We showed that five prolines in an N-terminal leucine repeat (LR) determine Rta's correct oligomeric state. The LR is not sufficient for Rta tetramerization, however, but functions in concert with the C-terminus of Rta.

Comparison of the gamma-herpesvirinae reveals a striking abundance of prolines as 18% of Rta's conserved amino acids. We hypothesize that proline-directed post-translational modifications of Rta could regulate Rta's function as a consequence of altering its multimerization. Two possible modifications of Rta are prolyl hydroxylation and proline-directed phosphorylation. We have found that Rta is highly phosphorylated *in vivo*, and some, but not all, of the Rta expressed in PEL cells contains hydroxyproline. Proline-directed phosphorylation can regulate protein structure by controlling binding to peptidyl-prolyl cis/trans isomerases (PPIases). The cellular PPIase Pin1 binds specifically to S/T-P motifs when serine or threonine is phosphorylated, and stimulates isomerization about the S/T-P peptide bond. In human oncogenesis, Pin1 overexpression deregulates many critical oncoproteins and tumor suppressors.

We demonstrate that Pin1 is expressed in latently-infected PEL cells, and its expression is induced by some, but not all, stimuli that reactivate KSHV. We show that the Rta protein contains 15 potential S/T-P motifs and binds directly to Pin1 *in vitro* and in infected cell lysates. Pin1 significantly enhances Rta-mediated transactivation of two viral promoters. Pin1's effect on Rta, however, is limited to a narrow window of concentrations, suggesting a rheostat-like influence on Rta's function. Indeed, in initial experiments, we observed little combinatorial effect of ectopic Pin1 on Rta-mediated reactivation of the virus. Interestingly, we found that ectopic expression of Pin1 alone induces Rta expression from the endogenous, latent viral genome. We are currently testing the hypothesis that Pin1 is a dose-dependent regulator of both Rta expression and function during KSHV reactivation. Experiments to test the significance of hydroxyproline in Rta's function are ongoing.

Mentor: David M. Lukac

Kalpana Dulal

Identification and analysis of human cytomegalovirus (HCMV) gene required for virus growth in vivo

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Human Cytomegalovirus (HCMV) is an opportunistic human pathogen that causes serious clinical illnesses in immunocompromised persons. The clinical strain of the virus, such as Toledo, contains an additional 15-kb region in its genome which is missing from the attenuated strains such as AD169. The attenuated strain has lost its ability to grow in the only available in vivo model system, severe combined immunodeficient mice with human tissue implant (SCID-hu), whereas the clinical strain grows well in this system. The inability of the attenuated strain to grow in mouse implants has been credited to the loss of the 15-kb region. Deletion of the 15-kb region from the Toledo virus impaired its growth in vivo whereas rescuing the region restored its growth. These findings imply that the gene essential for viral growth in vivo is located in the 15-kb region which encodes for 23 unique open reading frames (ORFs). The aim of this project is to identify the genes that are crucial for viral growth in vivo using a mutagenesis based approach. As an improvement for viral growth monitoring in vitro and in vivo, we have inserted a luciferase reporter gene in the Bacterial artificial chromosome (BAC) clone of the Toledo genome (ToledoLuc BAC). The virus made from this ToledoLuc BAC grows like its parental strain. Using the ToledoLuc BAC, we constructed a 15-kb deletion (15-kbD) and a 15-kb rescue (15-kbR) Toledo virus and studied their growth in vivo. As expected the 15-kbD virus did not grow in the mice implants where as the 15-kbR grew like wild-type. In order to narrow down the location of the critical genes, four smaller deletion clones each with 4/5 ORFs from the 15-kb region have been made. All the mutant viruses grew well in cultured fibroblast cells. One of the smaller deletion virus, deletion 4 (4D) showed severe growth defect in the mice model indicating that the genes required for viral growth is located within this region. To pin point the essential ORFs from region 4, three different mutants (UL132-UL128D, UL128-UL149D and UL149-UL151D) were constructed from the region 4. To our surprise all three mutants grew in the mice similar to the wild type virus. Our finding suggested an interesting phenomenon in HCMV gene functionality; one or more ORF have similar function in vivo.

Deletion 1 also showed growth defect in the mice implant indicating the genes located in the region also play some roles in viral growth the in vivo system. Mutant viruses with individual gene deletion from region 1 were also tested in the SCID-hu mice. Similar to the mutants from region 4, these individual gene deletion mutants also showed no growth defect in the mice. Here again, our result showed exciting finding about the group of genes working together and complementing each others function.

Mentor: Hua Zhu

Kevin Nguyen

Analyzing ARID1B as a Target to block Proliferation of Cancer Cells

Biomedical Sciences (Interdisciplinary), University of Medicine and Dentistry of New Jersey, GSBS, Newark

Analyzing ARID1B as a Target to block Proliferation of Cancer Cells

Kevin Hong Nguyen, Stephen Flowers, Fuhua Xu, and Elizabeth Moran

Background: SWI/SNF is an ATPase-powered complex required for chromatin remodeling, and therefore regulation of gene expression. SWI/SNF is increasingly recognized for its role in suppression of many different forms of cancer including breast, kidney, pancreas, and ovary. Our lab has cloned ARID1A, a subunit of the SWI/SNF complex, and shown that it is required for differentiation-associated cell cycle arrest. We identified the specific subunit ARID1A as a potential human tumor suppressor through screening of a human tumor tissue array. Recently, deep-sequencing projects have provided compelling statistical evidence that a range of human tumors of epithelial origin is deficient for ARID1A. Based on this information, we have considered the potential effects of down-regulating ARID1B. ARID1B is an alternative subunit to ARID1A in SWI/SNF. Our previous results showed that ARID1A and ARID1B play opposing role in cell cycle control. ARID1A is essential for normal cell cycle arrest, but ARID1B promotes expression of proliferation-related genes, most prominently c-myc. Hypothesis: ARID1B may be essential for proliferation of tumor cells where growth is still responsive to SWI/SNF control.

Experimental Approach and Controls:

1. Construct an adenovirus vector encoding shRNA sequences targeting ARID1B, as well as a control virus vector encoding a non-targeting “scrambled” shRNA sequence.
2. Knock down ARID1B in proliferating tumor cells (e.g. osteosarcoma cells), where SWI/SNF is still intact, and measure the cellular growth rate. Compare with a control tumor cell line not under SWI/SNF control due to complete loss of the core ATPase subunits.
3. If depleting ARID1B impairs the growth of osteosarcoma cells, we will expand testing to include epithelial cells deficient for ARID1A, where SWI/SNF is otherwise intact. Examples would be the renal carcinoma lines, CAKI-1 and CAKI-2.

Results: Depletion of ARID1B dramatically slowed the growth of all osteosarcoma cell lines tested, SaOS2, U2-OS and OHS-50. In a control tumor line cell line, HuTu80, which has escaped regulation by SWI/SNF, ARID1B depletion had no affect, supporting the conclusion that ARID1B is acting through SWI/SNF. Based on these findings, ARID1B is a possible therapeutic target for slowing the growth of a range of tumor cells driven by active SWI/SNF, particularly those depleted for ARID1A. In the near future, we plan to explore whether depleting ARID1B is sufficient to slow the growth of ARID1A-deficient cancer cells from tissue types including breast, kidney, pancreas, and ovary.

Mentor: Elizabeth Moran

Mahrukh Banday

Functional characterization of pseudo-CTD of RNA polymerase II in *Trypanosoma brucei*

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey, GSBS, Newark

The carboxyl-terminal end of all eukaryotic RNA polymerase II enzymes consists of a well recognizable domain called the Carboxy Terminal Domain (CTD). In yeast and mammals, the RNA polymerase II CTD contains tandem heptapeptide repeats, consisting of YSPTSPS, which orchestrate the essential co-transcriptional functions associated with mRNA maturation, including transcription initiation, elongation, capping, splicing and polyadenylation. These repeats undergo specific phosphorylation events, which generate a phospho-CTD code that regulates these co-transcriptional processes. In *Trypanosoma brucei*, RNA polymerase II lacks tandem repeats in its carboxyl terminus, replacing them with a non-repeated sequence termed as pseudo-CTD. The *T. brucei* pseudo-CTD is essential for viability of the organism and contains what seems to be a dynamic set of phospho-serine amino acids. Our hypothesis is that the pseudo-CTD contains a CTD-code, and by cracking this code we will understand the basic components of co-transcriptional events in these evolutionarily diverse organisms. We have established a versatile experimental setup in which cells are depleted of endogenous RPB1 (by tetracycline-regulated RNAi-mediated knock-down) and simultaneously supplied with an ectopic and tagged copy of RNAi-resistant RPB1. Using this system, we are testing RPB1 proteins that have multiple serine to alanine substitutions. Our study so far has indicated that specific sets of serine residues are indeed essential for trypanosome cell viability. We also have evidence indicating that the ectopic tagged copy of RPB1 gets incorporated into the RNA polymerase II transcription complex. Currently we are assessing which functions of RNA polymerase II are disrupted by these mutations

Mentor: Dr. Vivian Bellofatto

Michael Levandoski

Finding Intracellular Sites where mRNA Molecules Containing Premature Termination Codons are Recognized and Degraded

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Nonsense-mediated decay is a widely conserved process that removes messenger RNAs containing premature termination codons from the cell. In addition to providing a vital role in ridding the cell of potentially harmful proteins, nonsense-mediated decay has also been shown to reduce genetic noise by down-regulating a significant percentage of natural transcripts. Nevertheless, the location in the cell in which the premature termination codon containing mRNA is degraded is unknown. Using single-molecule fluorescence in situ hybridization, I will identify the subcellular locations of nonsense-mediated decay.

Mentor: Sanjay Tyagi

Mike Mosel

Generators of superoxide protect *Escherichia coli* from lethal stress

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The lethal action of many antimicrobials involves a cascade of reactive oxygen species (ROS), extending from superoxide through hydrogen peroxide to highly toxic hydroxyl radicals. Surprisingly, pretreatment of *Escherichia coli* with sub-inhibitory concentrations of metabolic generators of superoxide (plumbagin and paraquat) reduced rather than enhanced the lethal activity of quinolones, kanamycin, and ampicillin. These protective effects of low-level superoxide generators contrast with previous work in which presumably high levels of superoxide stimulated lethal activity. We propose that superoxide can either reduce or increase the effect of lethal stress depending on superoxide levels. Elucidating this dual function of superoxide is likely to be important for understanding bacterial stress responses to antimicrobials. Mutations in *soxS*, *marA*, and *katG* failed to block superoxide-mediated protection from antimicrobial lethality, indicating that the protection is neither a part of the major ROS-scavenging systems nor the Mar efflux-detoxification network. The protective effect of superoxide may involve increasing the size of bacterial subpopulations that are tolerant to multiple stressors.

Mentor: Drlica/Zhao

Olga D Gonzalez-Lopez

THE KAPOSÍ'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV) LYTIC SWITCH PROTEIN, RTA, STIMULATES DNA BINDING OF THE CELLULAR EFFECTOR OF NOTCH SIGNALING, RBP-JK, THROUGH HIGH AFFINITY INTERACTIONS WITH REPETITIVE CANT ELEMENTS IN PROMOTERS.

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We and others have demonstrated that reactivation of Kaposi's sarcoma-associated herpesvirus ((KSHV; Human herpesvirus-8 (HHV-8)) from latency in cultured B cells requires interactions between the viral lytic switch protein, Rta, and the cellular protein RBP-Jk. RBP-Jk is a monomeric 60kDa protein that is the nuclear target of the proto-oncogenic Notch signaling pathway. In the canonical model for Notch signaling, RBP-Jk specifies Notch's transcriptional targets by sequence specific DNA binding. In the absence of a Notch signal, DNA-bound RBP-Jk represses transcription. Our studies demonstrate that expression of essential genes during KSHV reactivation depends upon novel mechanisms for regulating Notch signaling. Although the KSHV genome contains at least 260 predicted binding sites for RBP-Jk, only forced expression of Rta, but not the Notch activating proteins NICD1 or Epstein-Barr Virus Nuclear Antigen (EBNA)-2, efficiently reactivates KSHV from latency. These and other observations challenge the assumption that RBP-Jk is constitutively bound to DNA in KSHV infected cells. We hypothesize that a novel mechanism determines Notch promoter selection during KSHV infection.

We previously showed that RBP-Jk is recruited to DNA by interactions with Rta. In the current study, we define the promoter requirements for formation of transcriptionally productive Rta/RBP-Jk/DNA complexes. We show that highly pure Rta footprints 7 copies of a novel repetitive element in the promoter of the essential KSHV Mta gene. We have termed this element the "CANT repeat." We demonstrate that Rta tetramers make high affinity interactions (i.e. nM) with 64 bp of the Mta promoter, but not single CANT units. The number of CANT repeats, their presence in palindromes, and their positions relative to the RBP-Jk binding site determines the optimal target for Rta stimulation of RBP-Jk DNA binding, and formation of ternary Rta/RBP-Jk/DNA complexes. DNA binding and tetramerization mutants of Rta fail to stimulate RBP-Jk DNA binding. Our ChIP assays show that RBP-Jk DNA binding is broadly, but selectively, stimulated across the entire KSHV genome during reactivation. We present a model in which tetramerization of Rta allows it to straddle RBP-Jk and contact repeat units on both sides of RBP-Jk. Our study provides insight into the molecular mechanism by which Rta stimulates RBP-Jk DNA binding, a novel level of regulation of the Notch pathway.

Mentor: David M. Lukac

Rinki Chauhan

The sigma factor network of *M. tuberculosis*

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The ability of *Mycobacterium tuberculosis* to persist for long periods of time is the major reason for the success of this pathogen. The bacterium has evolved strategies to face multiple stresses during the persistent stage. Accessory sigma factors play the most critical role during environmental adaptation and stress response in *Mycobacterium tuberculosis*. The exchange of the sigma factor subunit reprograms RNA polymerase to recognize and transcribe sets of genes expressing cellular functions that are critical for bacterial survival. We need to unravel regulatory interactions between sigma factors to understand the stress response of this pathogen. I have used two-plasmid based *E. coli* system to identify the direct interactions between accessory sigma factors. This approach has already been used to identify promoters recognized by sigJ. The test sigma factor is expressed under T7 promoter in first plasmid. The second plasmid is promoter probe plasmid, which carries the upstream promoter sequence of target sigma factor fused to reporter lacZ cassette. Beta-galactosidase activity expressed by promoter::lacZ cassette is measured to determine the effect of over-expression of a test *M. tuberculosis* sigma factor gene. I have optimized and established this method by demonstrating the involvement of sigE in the regulation of sigB, which is already known through previous reports. The outcome of this work will contribute towards reconstruction of the sigma factor network of *M. tuberculosis*, which will provide new targets for anti-tuberculosis drugs to prevent *M. tuberculosis* persistence.

Mentor: Marila Gennaro

Stacey Garcia

The Role of Poly(A)-Specific Ribonuclease in Stage-Specific mRNA Turnover in *Trypanosoma brucei*

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In trypanosomes, most protein-encoding genes are transcribed as part of long polycistronic pre-mRNAs that are subsequently processed into mature monocistronic mRNAs. Each open reading frame of the original pre-mRNA can exhibit different steady state levels, indicating that post-transcriptional regulation is important for gene expression. Deadenylation is often the rate-limiting event in regulating the turnover of cellular mRNAs in eukaryotes. Removal of the poly(A) tail initiates mRNA degradation by one of several decay pathways, including 5' to 3' exonuclease decay and 3' to 5' exosome-mediated decay. Poly(A)-specific ribonuclease (PARN) is a key deadenylase involved in regulating gene expression in mammals, *Xenopus* oocytes, and higher plants. Trypanosomatids possess three different PARN genes, PARN-1, -2, and -3. Here, we show that *Trypanosoma brucei* PARN-1 and PARN-3 are active deadenylases *in vitro*. Microarray data indicates that overexpression of PARN-1 and PARN-3 each down regulate a different subset of mRNAs. mRNAs affected by PARN-1 and PARN-3 overexpression include mRNAs encoding stage-specific coat proteins. qRT-PCR of stage-specific mRNAs confirms that PARN-1 overexpression increases the decay rate of these messages. Taken together, these data suggest that PARN-1 and PARN-3 are deadenylases that regulate stage-specific protein expression.

Mentor: Vivian Bellofatto

Wei Yang

Developing a Genetically-Encodable Biosensor for Imaging RNA in Live Cells

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey, GSBS, Newark

This project aims to develop a genetically encoded RNA based nucleic acid hybridization system to report the presence of specific RNAs in live cells. When the designed RNA probe is expressed inside a living cell, its probe sequence is able to hybridize to the RNA of interest and subsequently causes the formation of a structural motif in the probe. The structural motif, called aptamer, can bind tightly to a cell-permeable dye, which is non-fluorescent in its free form but become highly fluorescent when bound to its aptamer. The fluorescence signal coming from the restraint dye can be used as the indication for the presence of target RNA. Based on the photochemical and biological properties, we have selected and synthesized several small dye molecules. However, the screening of an aptamer, which possesses certain affinity and fluorescence increasing ability towards a selected dye, can be very challenging. We are now applying in vitro selection technology (Systematic Evolution of Ligand by EXponential enrichment, SELEX), combined with an in vivo fluorescence based screening system to isolate a dye-aptamer pair suited for our RNA system.

Mentor: Sanjay Tyagi

Department of Oral Biology

Anukriti Gupta

Ganeshnarayan Krishnaraj

Olga Korczeniewska *

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LYMPHOCYTE FUNCTION ANTIGEN-1 AND LEUKOTOXIN INTERACTION

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Lymphocyte Function Antigen-1 (LFA-1) is a beta-2 integrin expressed on the surface of human WBCs. LFA-1 undergoes an inducible conformational change from a non adhesive inactive to an adhesive active state, the latter mediating migration from blood vessels into tissues. Hematological malignancies are characterized by proliferation of WBCs that overexpress activated LFA-1. LFA-1 is known to be the specific target of Leukotoxin (LtxA), a major virulence factor of oral bacterium *Aggregatibacter actinomycetemcomitans* that causes aggressive periodontitis. LtxA targets primate WBCs migrated to the periodontium countering host response against the bacterium. Because of this natural specificity, LtxA was tested for its cytotoxic effect on THP-1 cells *ex vivo*, which were found to be very sensitive to LtxA. Humanized mouse models of leukemia (HL-60) showed prolonged disease free survival upon LtxA injection. Previous studies indicate preference of LtxA to activated WBCs compared to unactivated ones. Therefore, we wished to determine if this preference was due to LtxA specifically binding to active or open conformation of LFA-1. mAB24 was used as marker for active conformation of LFA-1 while CD11a (clone: HI1111) was used as a marker for inactive conformation of LFA-1. Preincubation of fixed THP-1 cells showing high levels of active LFA-1 (mAB24+) with LtxA led to decrease in mAB24 binding indicating that LtxA competes with mAB24 binding site on active conformation of LFA-1. In contrast, preincubation with LtxA had no effect on binding of CD11a mAB HI111 which recognizes only the inactive conformation of LFA-1. These results indicate specificity of LtxA for active LFA-1. The levels of active LFA-1 on healthy WBCs isolated from human blood were relatively low at baseline but increased upon stimulation with phorbol esters. This increase was more pronounced in granulocytes and monocytes. Treatment of human granulocytes and monocytes with LtxA *ex vivo* showed depletion of only active LFA-1 population.

Establishing this specificity is important to provide maximum therapeutic benefit targeting abnormal WBCs with active LFA-1 and sparing normal WBCs expressing inactive LFA-1 thereby minimizing immunosuppression.

Mentor: Scott C. Kachlany

Ganeshnarayan Krishnaraj

Human Salivary Cystatin SA exhibits antibacterial effect against the periodontopathogen, *Aggregatibacter actinomycetemcomitans*

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Among the various diseases in the oral cavity, Localized Aggressive Periodontitis is a particularly severe form of periodontal disease resulting in the early exfoliation of teeth, which is most commonly found in otherwise healthy young adolescents. It is associated with minimal local factors like plaque and calculus and therefore many cases of LAP remains undiagnosed till it reaches an advanced stage. *Aggregatibacter actinomycetemcomitans* (Aa), a gram negative coccobacillus, has been implicated as the primary etiologic agent of this form of disease. Aa forms extremely tenacious biofilms and along with other virulence factors like leukotoxin, cytolethal distending toxins is extremely potent at causing disease.

The human saliva possesses an array of proteins and factors that have a gamut of functions. Some of these include digestion, lubrication, buffering and are essential in maintaining the tooth and mucosal integrity. Research in the past few decades have focused on the protective role of saliva in preventing oral diseases like dental caries and periodontal disease. Some of the well documented proteins with significant antimicrobial properties include lactoferrin, histatin, lysozymes and immunoglobulins. Cysteine proteases comprise a group of proteolytic enzymes that cleave peptide bonds by the use of a reactive cysteine residue at the catalytic site. Some examples include endoproteinases like papain; lysosomal cathepsins like Cathepsin B, H and L. Cystatins are cysteine protease inhibitors and the salivary Cystatins including Cystatin S, SA and SN form a major part. Previously, Cystatins and sequences derived from Cystatin C have been shown to possess antimicrobial properties (Bjorck et al, 1989). Cystatin SA was purified from saliva of subjects that demonstrate antibacterial effect against Aa. The purified Cystatin SA was tested for its antibacterial effect against many organisms including Aa. The results show that Cystatin SA demonstrates significant antimicrobial effect against Aa in a dose dependent manner. It was also seen that sub lethal concentrations of Cystatin SA reduces the binding of the bacteria to Buccal Epithelial cells. Antibodies to Cystatin SA negated the effect of Cystatin SA. There is no previous report demonstrating antimicrobial effect of Cystatin SA against Aa and further studies to elucidate the mechanism of action are needed to understand the function of Cystatin SA in the oral cavity.

Mentor: Daniel H Fine

Olga Korczeniewska

Critical and quantitative review of literature to prioritize the assessment of genes for studying non-syndromic orofacial clefting

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Non-syndromic orofacial clefting has been studied intensively and numerous genes have been identified to be associated with the disorder. However the results of these studies were not always uniform and varied depending on clefting outcome and population studied. Therefore, a critical and quantitative review of orofacial clefting literature assessing the strength of published orofacial clefting association studies will provide a valuable resource summarizing up to date candidate gene findings relevant to the etiology of oral clefts. This literature review will allow for the identification of candidate genes that show the most supportive evidence of association with different clefting outcomes.

Objectives: To conduct a critical and quantitative review of literature in order to prioritize the assessment of genes most relevant in the etiology of orofacial clefting.

Methods: Pubmed literature search was conducted by means of specific search keywords ('cleft lip', 'cleft palate', 'orofacial cleft*', 'SNP*', 'polymorphism*', 'GWAS', 'genome wide association *'). The search yielded 394 articles 199 of which were reviewed as they corresponded to association studies of orofacial clefting.

Results: Following stringent selection criteria the review of over 5,000 orofacial clefting associations lead to the identification of 17 genes (ABCA4, C1orf107, C9orf156, CLEC4GP1, COL2A1, FOXE1, IRF6, JAM3, LOC728685, MAFB, MYH9, NAT2, PRSS35, SKI, SPATA17, SYT14, and WNT3) and one gene region (8q24) as candidates for studying associations with orofacial clefting as they provided most supportive evidence in the literature.

Conclusions: Non-syndromic orofacial clefting has been studied extensively and multiple genes have been implicated in the etiology of this disorder. However, to date causal mutations with known functional effects have not yet been identified and only about 14% of genetic contribution to orofacial clefting has been recognized. Implementation of technologies such as genome-wide association studies as well as sequencing will aid in the discovery of disease genes and identification of both gene-environment and gene-gene interactions to facilitate knowledge for risk counseling and development of preventive therapies.

Mentor: Scott Diehl

Yongyi Mei

Functional Mapping of *Aggregatibacter actinomycetemcomitans* Autotransporter Adhesin Protein---ApiA

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Actinobacillus putative invasin A (ApiA) is a trimeric autotransporter protein in periodontopathogen *Aggregatibacter actinomycetemcomitans* (A.a). Previous studies demonstrate that ApiA is a versatile virulence factor that acts as an adhesin, invasin, a factor for serum resistance, intercellular auto-aggregation of A.a and the inducer for cytokine release. The functional analysis of one ApiA mutant strain indicates that auto-aggregation domain is located near c-terminal membrane anchor domain. The missing c-terminal leads to abrogation of protein trimeric formation, strongly reduced protein surface display, autoaggregation, and adhesion properties. Further, we will identify the binding domain that mediates species-specific binding of A.a to buccal epithelia cells.

Mentor: Daniel H. Fine

Department of Molecular Pathology and Laboratory Medicine

Mili Mandal *

Chingiz Underbayev

Crystal J. Dicosmo

Dante Descalzi

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Maternal immune stimulation during pregnancy facilitates prenatal immuno-developmental changes leading to a pro-inflammatory phenotype in offspring

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Epidemiological studies show that infection during pregnancy increases risk of neurodevelopmental disorders in children. In rodents, injection of pregnant dams with infectious pathogens or agents that mimic viral or bacterial infections also leads to neurological, behavioral, and immunological abnormalities in their offspring. Our overall hypothesis is that in addition to genetic susceptibility, maternal immune stimulation during pregnancy acts as an environmental trigger that initiates immune-mediated mechanisms resulting in manifestations seen in some children with Autism Spectrum Disorders.

C57BL/6 females and males were mated. On gestational day 12, pregnant dams were injected i.p. with PBS or poly(I:C), and scored for sickness behavior. Sera and amniotic fluids from dams were tested for the presence of cytokines, and lymphocyte phenotype/functional analyses were performed on their offspring. In addition, offspring were given a second immune stimulus, either i.p. zymosan injection to induce an antigen non-specific acute inflammatory response or MOG35-55 induced antigen-specific experimental autoimmune encephalitis (EAE). Offspring were analyzed for qualitative and quantitative differences in their responses to these immune stimuli.

After 2 hrs, poly(I:C)-injected pregnant dams showed a significant increase in sickness behavior and in the levels of pro-inflammatory cytokines in sera and amniotic fluids compared to PBS-injected pregnant dams, indicating successful maternal response to poly(I:C). FACS analysis of activated spleen cells from offspring of poly(I:C)-injected dams (vs. PBS-injected dams) showed preferential differentiation toward Th17 cells. Offspring of poly(I:C)-injected dams also showed heightened acute inflammatory responses as seen by significantly higher number of total peritoneal exudate cells (predominantly neutrophils), and significant increases in pro-inflammatory cytokine levels in sera and peritoneal cavity fluid after zymosan injection. In addition, these offspring showed an exacerbated response to MOG35-55 and displayed significantly earlier onset and higher frequency of clinical symptoms of EAE. These results demonstrate that offspring of poly(I:C)-injected dams possess a pro-inflammatory phenotype, thus exhibiting more robust innate and adaptive responses upon postnatal immune stimulation. Such “fetal programming” of offspring is likely to be mediated by the cytokines produced as a result of in utero stimulation.

Mentor: Nicholas M. Ponzio

Chingiz Underbayev

Generation of induced pluripotent stem cells from New Zealand Black mouse somatic cells: exploring the role of microRNA-15a/16-1 defect in B-cell lymphogenesis

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Chronic lymphocytic leukemia (CLL), a malignancy of the CD5⁺ B cell, is the most common leukemia to affect adults in the Western world. More than 50% of CLL cases exhibit a deletion within the 13q14 chromosomal region containing microRNAs miR-15a and miR-16-1, which are down-regulated in a sub-population of patients with B-cell CLL. New Zealand Black (NZB) mouse is a de-novo model of CLL that has been studied extensively as a model of both autoimmune diseases, such as systemic lupus erythematosus (SLE), as well as B-cell lymphoproliferative disorders. This mouse exhibits a T→A point mutation six bases downstream from pre-miR-16 region on chromosome 14, similar to the C→T point mutation seen in human CLL. The usefulness of pluripotent stem cells such as embryonic stem (ES) cell and induced pluripotent stem (iPS) cells is partly due to their amenability to efficient gene manipulation. Homologous recombination between genomic and the exogenous DNA is a very inefficient event, but it takes place in ES/iPS cells with relatively higher efficiency than it does in other cell types. In this way mice with a variety of modifications such as null and point mutations, chromosomal rearrangements and large deletions have been generated. Unfortunately, NZB mouse appears to be refractory to true ES cells derivation. Here we report an induction of induced pluripotent stem-like cells from NZB spleen stromal fibroblasts by means of lentiviral delivery of three factors: Oct4, Sox2 and Klf4 in a single polycistronic vector. After 4 weeks in culture medium supplemented with small molecules (GSK-3b and MEK inhibitors) we were able to see ES-like colonies which stained positive for alkaline phosphatase and SSEA-1 surface antigen. Colonies have been picked and expanded for further analysis. RT-PCR assay showed the expression of basic endogenous pluripotency genes such as Oct4, Sox2, Klf4, cMyc and Nanog. The pluripotent capacity of NZB iPS cell was further successfully confirmed by teratoma formation assay in NOD-SCID mice. These iPS cells will be used to correct miR-15a/16-1 mutation and deletion by homologous recombination followed by the in vitro differentiation into the B-lineage cells. This work will help uncover the unknown mechanisms of CLL development and shed the light on the potential usefulness of miR-15a/16-1 as a therapeutic target in CLL patients.

Mentor: Elizabeth Raveche

Crystal J. DiCosmo

The role of macrophage migration inhibitory factor (MIF) in breast cancer cell movement

*Crystal J. DiCosmo, Frederick D. Coffman, Stanley Cohen and Marion C. Cohen.
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Although macrophage Migration Inhibitory Factor (MIF) was one of the first cytokines to be identified, its role in inflammatory and immune responses received little attention for many years due to difficulties in both cloning and the biological assays. However, recent studies indicate MIF plays important roles in many disease states including tumorigenesis. It was originally defined by its ability to inhibit migration of macrophages utilizing the capillary tube and agarose microdroplet assays. In our studies, we used the transwell migration assay and found that recombinant human MIF (hMIF) exhibited inhibitory biological activity against a series of human breast cancer cell lines of increasing malignancy and metastatic potential.

The cytoskeleton is a critical target of signals regulating or induced by cell-matrix interactions, and the family of small GTPases has been implicated as key modulators of cytoskeletal dynamics that occur after an adhesion event. Therefore, we have begun to investigate the role of the small GTPases in MIF-mediated migration inhibition. The results of preliminary experiments using a G-LISA-based activation assay support a key role for these molecules in MIF-mediated inhibition of cell movement. We have found that 1,000 ng/mL hMIF has an impact on the activities of RhoA, Rac1, and Cdc42 after addition to breast cancer cells. Future studies will investigate whether or not blocking methodology is capable of restoring a normal migration phenotype in MIF-treated cells. We will also investigate the structural changes that accompany changes to the small GTPases in human breast cancer cell lines.

Mentor: Marion C. Cohen

Dante Descalzi

The histone deacetylase inhibitor, Trichostatin A, decreases TLR-mediated cytokine production in human plasmacytoid dendritic cells (pDC) by negatively affecting IRF-7 phosphorylation and nuclear translocation

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pDC produce high levels of IFN- α and TNF- α upon TLR7/9 stimulation and express high constitutive levels of the transcription factor IRF-7, which is required for IFN- α induction. Histone deacetylases (HDAC) are enzymes shown to regulate protein function by removing acetyl groups from nuclear and cytoplasmic targets. HDAC inhibitors are used clinically as cancer therapeutics and are being considered as potential anti-inflammatory agents. Our purpose was to characterize the effects of Trichostatin A (TSA), a class I/II HDAC inhibitor, on pDC cytokine production and IRF-7 protein regulation and activation. TSA treatment strongly inhibited the induction of both IFN- α and TNF- α by pDC upon stimulation with CpG-A, HSV-1, HIV-1, or Flu, as shown by intracellular flow cytometry and ELISA. TSA treatment did not induce pDC apoptosis, nor did it negatively affect cell number, arguing against general toxicity of the drug. Interestingly, although basal IRF-7 levels were not affected by TSA, upregulation of pDC IRF-7 protein expression in response to rIFN- α was inhibited. Moreover, TSA treatment inhibited virus-induced IRF-7 activation, as demonstrated by BD Phosphoflow, and nuclear translocation of IRF-7, as demonstrated by ImageStream imaging flow cytometry. These findings suggest a potential impact of acetylation on the activation and protein expression of IRF-7 in pDC and highlight a possible mechanism of cytokine inhibition by TSA in these cells.

Mentor: Patricia Fitzgerald-Bocarsly

David Mwangi

Assessment of the role of CD4 and CD2 in plasmacytoid dendritic cells in response to viral infection.

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pDC secrete type 1 interferons and pro-inflammatory cytokines upon viral stimulation through either TLR7 or TLR9. The numbers and function of pDC are impaired in HIV infection, but the mechanism of this deficiency is not fully understood. pDC express CD4, CXCR4 and CCR5, the receptor and co-receptors for the HIV envelope protein, gp120. A subset of pDC expresses CD2. The functional significance of these molecules on pDC in HIV infection is unknown. We hypothesize that CD4 is involved in DC:DC or TC:DC communication that leads to modulation of pDC function. Our data demonstrate that CD4 cross-linking inhibits activation of pDC as evidenced by a decrease in HSV-1-activated CD40 and HLA-DR expression and translocation of NF- κ B to the nucleus. We also hypothesize that CD2 subsets may differentially interact with TLR9 and TLR7 agonists and viruses. Our data shows that CD2+ pDC express higher levels of IP10, KI67, PDL1, CXCR4 and TLR7 while the CD2- subset shows higher levels of IL-6. The two subsets express comparable levels of IFN- α . CXCR3 and CCR5 when stimulated with HIV-1. CD2 expression does not appear to influence endocytosis and micropinocytosis, suggesting that differential uptake of virus may not account for the functional differences between the subsets. Future studies will seek to understand the significance of these findings with respect to activation of pDC and their potential interactions with HIV-1.

Mentor: Patricia Fitzgerald-Bocarsly

Jing Deng

The role of autophagy and amphisome formation in virus recognition and virus-stimulated IFN- α production by human plasmacytoid dendritic cells (pDC).

Jing Deng, Sukhwinder Singh, Jihong Dai and Patricia Fitzgerald-Bocarsly, University of Medicine and Dentistry of New Jersey – New Jersey Medical School and GSBS, Newark, NJ.

pDC secrete large quantities of IFN- α in response to viral nucleic acid stimulation via TLR7/9. However, little is known about virus trafficking in pDC after entry. To investigate the subcellular trafficking of viral particles, we examined intracellular events after viral stimulation. The formation of double-membrane autophagosomes was observed by EM within 2hr stimulation of pDC with HSV, Flu, HIV-1 or CpGA. Class I/III PI3K inhibitors 3-methyladenine, wortmannin and LY294002 significantly suppressed virus-induced IFN- α production at an early stage. However, using Amnis® imaging flow cytometry, we found that uptake of HSV-GFP was not inhibited, suggesting virus-induced autophagy rather than uptake was specifically blocked by PI3K inhibitors. To further investigate events related to virus-induced autophagy, we studied the colocalization of HSV-GFP particles, early endosomal marker CD71 and autophagosomal marker LC3B. CD71 co-localized with LC3B after pDC stimulation with virus or CpGA, indicating amphisomes, which are autophagic vacuoles formed from fusion of autophagosomes and endosomes, were induced. With chloroquine, an inhibitor of endosomal fusion and acidification, there was accumulation of the internalized HSV-GFP particles and amphisomes. Collectively, our studies demonstrate that amphisomes are induced by virus or TLR9 ligand stimulation in pDC. Further studies will be conducted to characterize the role of these amphisomes in virus-induced IFN- α production. (The 98th AAI Trainee Award Recipient)

Mentor: Patricia Fitzgerald-Bocarsly

Meher Patel

Up-regulation of programmed death ligand 1 expression on human plasmacytoid dendritic cells by viral stimulation

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The negative costimulatory molecules Programmed Death-1 (PD-1) and its ligand Programmed Death Ligand -1(PD-L1) negatively regulate the function of exhausted CD8 T cells in chronic HIV-1 infection leading to suppression of specific anti-viral T cell responses. Blockade of this pathway has been shown to reconstitute T cell function and augment antiviral immune responses. In HIV-infected individuals, increased PD-L1 (B7-H1) expression on APCs correlates directly with viral load, indicating a potential effect of the virus on PD-L1 expression. However, the mechanisms of PD-L1 up-regulation in these settings are not well understood. In this study, we investigated how PD-L1 expression is regulated in human plasmacytoid dendritic cells (pDCs). The pDC is a dynamic immune cell that can influence both innate as well as the adaptive arm of the immune system. In HIV-infected individuals there is a marked decline in pDC numbers as well as function. We found PD-L1 expression on pDC was up-regulated with different viral stimulations including HSV, Sendai virus, Influenza virus and both live and AT-2 inactivated HIVMN. Different viruses up-regulated PD-L1 but not to the same extent. HSV, Sendai virus and Influenza virus were better inducers of PD-L1 expression than HIV. Furthermore, we observed that LPS enhanced HIV-induced PD-L1 expression on pDC, suggesting potential involvement of LPS in the up-regulation of PD-L1 in chronic HIV-1 infection. The inflammatory cytokines elevated in HIV infection, TNF- α and IL-6, had no influence on PD-L1 expression. IFN- α treatment up-regulated PD-L1 expression on pDC, indicating that up-regulation of PD-L1 on pDC was specifically a primary response to viral stimulation. Taken together, our results suggest a potential role for up-regulation of PD-L1 expression on pDC in HIV-induced immune exhaustion.

Mentor: Patricia Fitzgerald-Bocarsly

Piotr Pierog

Toxoplasma gondii infection of human pDC inactivates TLR7 and -9 mediated induction of IFN- α and TNF- α in response to HIV and HSV

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pDC are the major producers of IFN- α , an antiviral cytokine involved in control of HIV replication, while *T. gondii* is a life-threatening opportunistic infection in AIDS patients. In this study, we investigated pDC responses in the context of virus and *T. gondii* co-infection. *T. gondii* invaded but did not induce IFN- α or TNF- α in human pDC. However, Class I, II and III strains of *T. gondii* inhibited IFN- α and TNF- α in response to HSV and HIV. Within the *T. gondii* exposed pDC population, IFN- α production was inhibited only in cells infected by *T. gondii*, whereas TNF- α was inhibited in both the infected and to a lesser extent, uninfected pDC. *T. gondii* inhibited neither uptake of GFP-HSV nor recruitment of TLR9 to EEA1+ endosomes. Using imaging flow cytometry we found that virus-induced nuclear translocation of IRF-7 was abolished by the parasite. Taken together, these data indicate that the block of the intracellular signaling cascade by *T. gondii* occurs downstream of TLR9 recruitment but upstream of IRF-7 translocation. Unexpectedly, *T. gondii* did not inhibit HSV-induced nuclear translocation of NF- κ B. We identified that Rop16, a parasite-derived kinase, is partially responsible for IFN- α and TNF- α inhibition. Additionally, *T. gondii* altered the virus-induced maturation profile of pDC. These findings suggest a novel mechanism of inhibition of TLR signaling by *T. gondii* and suggest potential negative consequences of HIV/*T. gondii* co-infection.

Mentor: Patricia Fitzgerald-Bocarsly

Siddha Kasar

Characterization and Manipulation of NZB miR15a/16-1 loci for CLL Therapy

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Chronic Lymphocytic Leukemia (CLL) is a chronic lymphoid malignancy characterized by accumulation of mature CD5+ B-cells in peripheral lymphoid organs, bone marrow and peripheral blood and is incurable with conventional therapy. We have previously shown that similar to human B-CLL cells, NZB mice (de novo mouse model of CLL) have 50% less level of miR15a/16-1 that is associated with a mutation and deletion in the miR15a/16-1 region. The NZB loci lead to a processing defect inhibiting the conversion of pri-miR15a/16-1 to pre-miR15a/16-1. Moreover, we hypothesized that exogenously increasing its expression would lead to increased apoptosis in tumor cells. Using a lentiviral delivery system, we were able to stably increase the level of these two microRNAs in LNC (NZB derived B-CLL cell line). The apoptosis observed in miR-GFP transduced LNC was twice as much as in LNC transduced with GFP lentivirus (control). In addition to the miR defect, B-CLL cells have a very high level of B-cell specific activator protein (BSAP) and this prevents their terminal differentiation. Recent reports have suggested that BSAP and miR15a/16-1 form an autoregulatory loop. We found that the level of the two oncoproteins BSAP and c-Myb were reduced in the miR-GFP transduced cells as compared to the control. Alternatively, we found that the miR defect can also be corrected by knocking down BSAP (using RNAi) leading to a cell cycle arrest. The second strategy is clinically relevant, since developing a BSAP inhibitor is more feasible than systemic miR delivery. Based on our findings we propose that targeting BSAP could be used as a novel therapeutic strategy for B-CLL.

Mentor: Elizabeth Raveche

Temitayo Awoyomi

Human plasmacytoid dendritic cells (pDC) sequentially up-regulate and co-express functional receptors and IFN- α in response to HIV and HSV stimulation.

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Human plasmacytoid dendritic cells undergo functional and phenotypic changes upon viral stimulation. These changes lead to IFN- α production and a coordinated modulation of functional receptors and cytokine production crucial for antigen presentation, T cell activation and trafficking. We investigated whether the same pDC that produce IFN- α express receptors involved in antigen presentation or whether these are functions of distinct subsets. Using flow cytometry and SPICE® analysis, we observed not only time- and stimulus-dependent activation profiles in these activated pDC, but also their ability to concurrently express multiple receptors important for their function as APCs on HIV-1 and HSV-1 stimulation. Kinetics of pDC activation were slightly slower with HIV than with HSV stimulation. CD4, CD80, CD83, CD86, PD-L1, MHCII, CCR5 and CCR7 were up-regulated concurrently with IFN- α and TNF- α in a pDC subpopulation, CXCR4 was recycled and CD62L was shed in this same subpopulation. We found subpopulations of pDC that expressed IFN- α and activation markers either singly or together. From the phenotypic progress over time, we hypothesize that these represent pDC that start producing IFN- α , then co-express IFN/activation markers, then go on to become fully mature. Further studies will tease apart interactions between these subsets and other cells of the immune system, especially in disease manifestations like HIV that specialize in suppressing and altering pDC function and phenotype.

Mentor: Patricia Fitzgerald-Bocarsly

Xiangwen Chen-Deutsch

Changes in the MAPK expression profile in AML cells ex vivo associated with differentiation induced by 1,25-dihydroxyvitamin D3 and its analogs PRI-1906 and PRI-2191

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The physiological form of 1,25-dihydroxyvitamin D3 (1,25D) has well-established anti-cancer activity, but its potential clinical use is limited by hypercalcemia. Efforts to overcome this difficulty include structural modifications of 1,25D, and enhancement of its activity by other compounds such as antioxidants. The analogs PRI-1906 (24-ene-1,25-dihydroxyvitamin D2) and PRI-2191 ([24R]-1,24-dihydroxyvitamin D3) had greater activity on AML cell lines than their congeners, but their potency on AML blasts was not entirely clear. We therefore studied different FAB subtypes of blood specimens obtained from AML patients. Monocytic differentiation markers CD11b and CD14 increased when the cells were treated with 1,25D, or its analogs PRI-1906 and PRI-2191 alone, and when combined with carnosic acid, a plant antioxidant. Concurrently, G1 arrest increased and proliferation index decreased as determined by cell cycle analysis of propidium iodide stained cells. Western blots showed increased levels of phosphorylated MAPK proteins including HPK1-JNK pathway. Blasts with three different subtypes of AML (M2, M3 and M5) were exposed to 1,25D or PRI-1906, PRI-2191, and RT2-PCR arrays were used to investigate the mRNA profile of the MAPK signaling pathways. While every patient's specimen had a unique profile, common features included an increase in p38delta signaling, and downstream MAPK transcription factors such as c-Jun showed a large increase in mRNA expression. We conclude that analogs of 1,25D, PRI-1906, and PRI-2191 induce efficient cell arrest and differentiation of AML cells ex vivo, and carnosic acid can enhance their effect. As previously shown for 1,25D, MAPK signaling network is an important regulator in this process. These compounds may be considered as candidates for chemoprevention/ differentiation therapy of AML.

Mentor: George P. Studzinski

Yao Yuan

Cytokine-induced interplay between miRNAs, lymphoid subpopulations and lupus in NZB/NZW F1 murine model

Department of Molecular Pathology and Immunology, University of Medicine and Dentistry of New Jersey, GSBS, Newark

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease and the specific cause remains unknown, however, B and T cell abnormalities are considered to play central roles in the pathogenesis. IFN- α and IFN- γ both have well defined positive effects in lupus disease development and, by delivering exogenous IFN- α to NZB/W F1 mice, accelerated lupus manifestations. Yet the effects of IFN- λ , a type III interferon sharing partially common signaling pathways with IFN- α , remains unknown in lupus. In this study, we treated B/W mice with IFN- α or IFN- λ for a 12-week period. The IFN- α treated group, but not the IFN- λ group, developed much earlier and more severe lupus-disease than others. Moreover, combination treatment with both cytokines enhanced the effects of either single treatment. By flow cytometry, at the time when only the IFN- α treated group developed proteinuria and other groups showed no proteinuria, we were able to identify that IFN- α treated mice showed a pronounced decrease in regulatory B cells (B10). In contrast, the IFN- λ treated group did not show a similar effect but rather an expansion of B10 cells. However, at a later time when all other groups developed proteinuria, IFN- λ treated group also showed a decrease in B10 cells, comparing to PBS treated control group. Furthermore, we wanted to investigate whether or not, as consequences of IFNs treatment, any specific microRNAs could be responsible for the cell type changes. Results showed miRNAs changes in accordance to disease status of B/W mice, a signature was found in B/W mice with proteinuria, plus, there is a pattern difference between B/W mice and non-autoimmune C57 mice. However, no characteristic miRNA changes were detected with the different IFNs treatment, although proteinuria was accelerated by IFNs. To validate the result, we selected miR-15a, which is highly elevated in diseased B/W and measured its expression in individual samples in the same group. Results were consistent with the PCR array. Thus, it is likely that miRNA alterations following IFN treatments may drive the disease acceleration in the lupus-prone B/W mice.

Mentor: Elizabeth Raveche

NOTES