RUTGERS

Graduate School of
Biomedical Sciences

2013 GSA Research Symposium

GRADUATE STUDENT ASSOCIATION

Welcome to the 2013 Graduate Student Association Research Symposium

We would like to extend a warm welcome to all attendees of the 2013 Graduate Student Association Research Symposium!

This event marks our 20th annual Symposium at the Graduate School of Biomedical Sciences at Newark and our first Symposium as a division of Rutgers Biomedical and Health Sciences. Every year, our Symposium gives our students the unique opportunity to learn about ongoing research projects in other laboratories as well as the chance to interact and exchange ideas with their colleagues. The poster sessions and oral presentations feature the wide range of exciting research our students are dedicated to—from small molecules to proteins, to cells, to behavior. In addition to the contributions of our own graduate researchers, we are proud to host this year's plenary speaker, Dr. Stefan Feske from the Department of Pathology at the New York University School of Medicine, who will enlighten us on his research with a talk entitled "Molecular regulation of CRAC channels and their role in immune responses".

The annual Symposium is a very special occasion for us all—a celebration of our achievements, and we would like to thank all of our students, faculty, and staff at the Graduate School of Biomedical Sciences for their help and support in making our event a success year after year.

Again, welcome, and we hope that you find the 2013 Graduate Student Association Research Symposium informative and enjoyable!

The Graduate Student Association Executive Board
Stephanie Veerasammy, President
Rick Gordan, Vice President
Courtney Rella, Secretary
Jennifer DeCotiis, Treasurer

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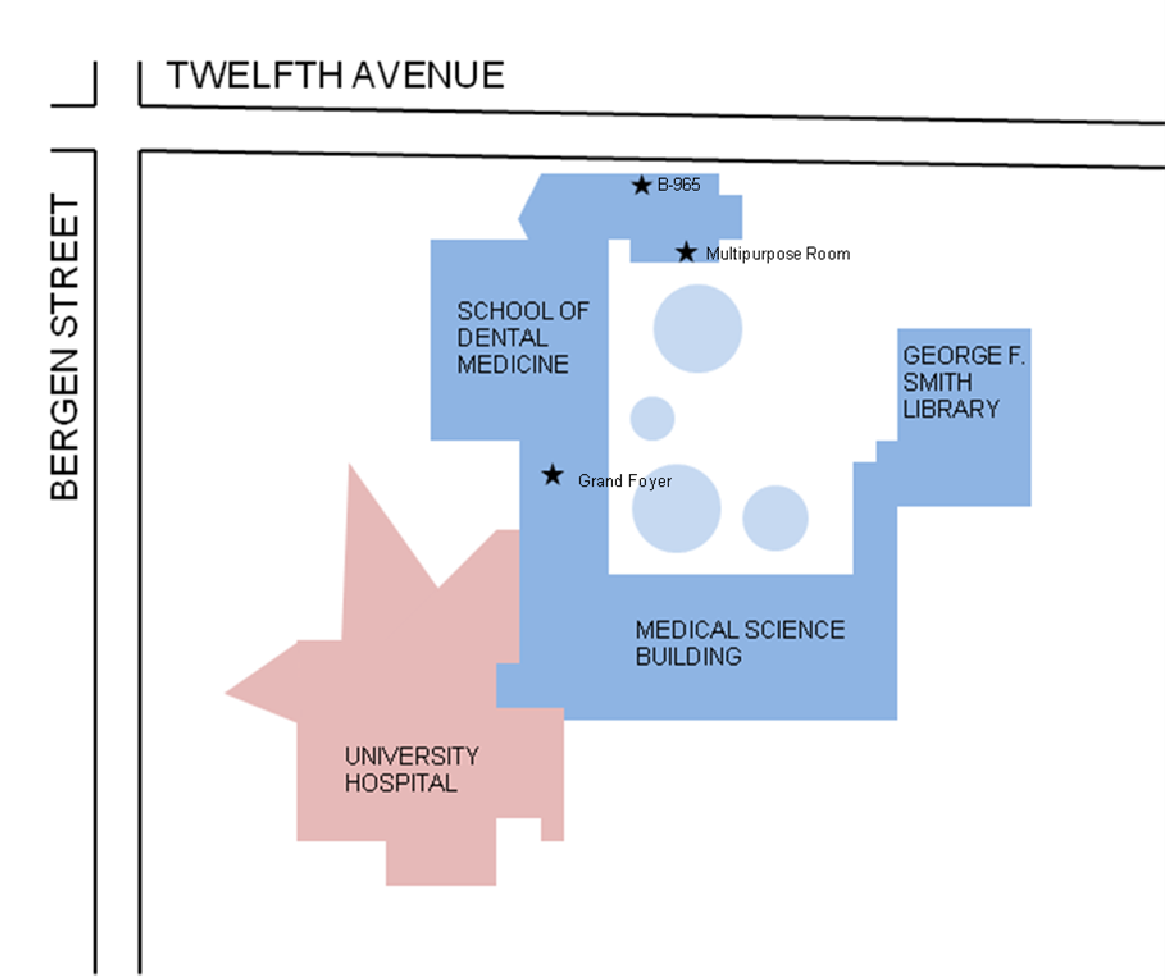
Event Map

Medical Science Building & School of Dental Medicine
Rutgers Health Sciences Campus at Newark



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Rutgers Graduate School of Biomedical Sciences—Newark

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Abstracts

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Analysis of the combination of Paired Associative Stimulation & Virtual Reality in rehabilitation of the upper extremity post-stroke

Ian Lafond
4th Year MD/PhD Student
Mentor: Sergei Adamovich, PhD
Department: Rehabilitation & Movement Sciences
Poster N^{O}01

The motor and neurological consequences of stroke are a major health concern in the United States, affecting almost 800,000 people per year. The intricacy of neuromotor control required for hand function as well as the variation in recovery of manipulative abilities post-stroke, makes rehabilitation of the upper extremities, especially the hand, extremely challenging. The goal is to investigate targeted therapeutic interventions that facilitate rehabilitation and motor recovery through plasticity-mediated therapies. We have combined several aspects of therapeutic interventions that utilize virtual reality (VR) technology interfaced with transcranial magnetic stimulation and electrical stimulation of the hand/ arm & arm/hand robotics to provide repetitive and intensive sensorimotor training needed to promote neuroplasticity and functional motor recovery after stroke. Our study of 17 healthy subjects shows that an optimal protocol combines single pulse TMS with single pulse peripheral stimulation. MEP amplitude changes were found to be the largest and most significant in this group, signifying an increase in the cortico-motor excitability and thus, brain-muscle communication. This study will serve as the basis for using this paradigm on individuals affected by stroke.

Mobility Rehabilitation using Stepping

Robbie Gosine
5th Year PhD Student
Mentor: Judith Deutsch, PT, PhD, FAPTA
Department: Rehabilitation & Movement Sciences
Poster N^{O}02

Our research investigates the use of Virtual Reality Games (VRGs) as a rehabilitation tool to promote mobility and balance using stepping. Conditions such as age, injury and disease can result in a reduced ability to perform simple stepping. Various investigators have reviewed changes in the ability to control the lower limbs due to these ailments and have found that functional motor control can be developed or improved with simple stepping in certain cases. The simplicity of stepping is important as it is a task that is very familiar, relatively safe and can be done under limited supervision.
One method that is available to implement a VRG for stepping is the Microsoft Kinect® camera system (MKCS). It been investigated as a rehabilitation tool by various research groups with encouraging results. Unlike other COTS devices, the MKCS is compatible across major operating systems such as Windows 7 and can be developed for a wide variety of uses making it portable, easily implemented and readily customized for specific applications.
Currently, we have developed a Beta VRG using the MKCS with built in stepping protocols that is user friendly and custom designed for the patient. While it is a game, it readily captures key sets of step data for later analysis – speed, distance, and velocity, to aid the clinician in planning the patient's therapy. We have done preliminary validation of the VRG and the MKCS with respect to the above data sets with very good results. A user study is scheduled to follow shortly.

Increased motor output is associated with M1 motor map expansion during isometric finger contraction

Mathew Yarossi
3rd Year PhD Student
Mentor: Eugene Tunik, PhD
Department: Rehabilitation & Movement Sciences
Poster N^{O}03

Precise control of finger movement is vital for activities of daily living and relies on finely graded contraction of agonist-antagonist muscles. This is not trivial from a neural control perspective because in the motor cortex (M1), corticospinal neurons innervating muscles that are movers of individual fingers are intermingled with those innervating multi-finger complexes. Previous studies measuring motor evoked potentials (MEP) with transcranial magnetic stimulation (TMS) to assay corticomuscular excitability, have reported increased MEP amplitude in synergistic muscles, and decreased MEP amplitude in relaxed nonsynergistic muscles during neighboring muscle movement. One possibility is M1 muscle fields are akin to visual receptive fields, and use a mechanism similar to surround inhibition (SI) to achieve the desired activation. Empirical data suggest SI in M1 may be associated only with low motor output such that decreases in MEP size of nonsynergistic muscles is diminished when a contraction is maintained or forces of >40% of maximum voluntary contraction (MVC) are required. To examine the relationship between SI and motor output, we quantified the amplitude distribution of TMS-evoked M1 maps of intrinsic hand and long forearm agonist-antagonist muscles during isometric contractions at graded force levels. Comparison of motor maps across force levels revealed an active expansion of excitable area with minimal change in position of the hotspot. This was evident for both the FDI and long forearm muscles in all subjects. Our data suggest that increased motor output is associated with expansion of cortical activation involving multiple muscles, perhaps relates to a loss of surround inhibition.

Frontal-Subcortical Function and Use of Assistive Speech Devices for Aphasia

Amit Chaudhari
4th Year MD/PhD Student
Mentor: A. M. Barrett, MD
Department: Physical Medicine & Rehabilitation
Poster N^{O}04

Authors: Amit Chaudhari, B.A.; James S. Maniscalco, M.A.; Jeffrey Zhang, PhD.; Darlene Williamson, M.A. CCC-SLP, Uri Adler, M.D., A.M. Barrett, M.D.

Objective: To identify neuropsychological predictors of successful assistive device use in aphasia.

Background: Assistive speech devices (ASDs) may augment communication efficacy in aphasia. However, patients may have difficulty using a mobile device, which requires diverse mental abilities including cognitive motor planning, concentration, visuospatial orientation and sequencing. We wished to evaluate whether performance on specific neuropsychological tests could predict device use success, thereby assisting clinicians in identifying ASD candidates.
Methods: 20 people with aphasia (60.6 ± 14.3 years) completed 9 neuropsychological tasks, including Finger Tapping Test (FTT), aphasia-adapted Frontal Assessment Battery (FAB), Test of Oral and Limb Apraxia (TOLA), Western Aphasia Battery (WAB), Behavioral Inattention Test (BIT), Communicative Effectiveness Index (CETI), Catherine Bergego Scale (CBS), Naturalistic Action Test (NAT), and Neuropsychological Assessment Battery (NAB). All patients were trained to use an ASD (O'Brien Technologies Survivor Speech Companion System) followed by 7 days' unrestricted home use. Then, each participant completed 3 device-based communication tasks (use the device to tell your marital status, give directions to Kessler Foundation, and ask for a glass of water). We performed a Stepwise Discriminant Function Analysis to evaluate how pre-trial tests performed in grouping High (>80%) vs. Low (<80%) scorers at device-based communication. We also conducted a factor analysis to explore the underlying structure of the groups' neuropsychological test scores.
Results: The Frontal Assessment Battery (FAB) was the only significant predictor of device use success (Wilk's Lambda=0.713, F=6.856, p=0.018, 28.7% variance), correctly classifying 80% of High/Low scores. A Factor Analysis indicated that a factor including the FAB, WAB, BIT, NAB and TOLA explained 45% of variance in overall neuropsychological performance (eigenvalues>1).
Conclusions: Identifying people with aphasia who can benefit from an assistive speech device is vital to prescribing an ASD. Here, aphasia severity was not predictive, but an aphasia-adapted version of the Frontal Assessment Battery predicted device success. These results show that frontal cognitive assessment may be needed in standard ASD assessment. Further research to identify which skills assessed by the FAB best predict device use success, and their relation to other neuropsychological deficits in aphasia, are indicated.

The effects of deployment-related exposures on mitochondrial dysfunction in Iraq/Afghanistan Veterans

Yang Chen
3rd Year PhD Student
Mentor: Michael Falvo, PhD
Department: Physical Medicine & Rehabilitation
Poster N^{O}05

Background: Military deployed to Iraq and Afghanistan are likely to have been exposed to high levels of air pollution during their deployment. Inhalational exposures, such as benzene and airborne particulate matter, have been shown to trigger oxidative stress-induced mitochondrial dysfunction. However, no studies have considered the effects of deployment-related exposures on mitochondrial dysfunction in deployed Veterans.
Objectives: Our goal is to investigate whether deployment-related exposures are associated with mitochondrial DNA copy number (mtDNAcn), an index of mitochondrial DNA damage and dysfunction.
Methods: We recruited 20 Veterans deployed to Iraq and Afghanistan. The deployment-related exposures were analyzed via self-reports of individual Veterans, based on 'Deployment Air Respiratory Exposures (DARE) Questionnaire'. DARE questionnaire was developed to rank the frequency, duration and intensity of different categories of exposures (sand and dust, smoke from burning trash, exhaust fumes and industrial air pollution). Veterans were assigned to HIGH and LOW exposure groups based on the rank of peak intensity of deployment-related exposures. Smoking history was also calculated in both groups. Relative mtDNAcn was determined by real-time polymerase chain reaction in saliva DNA obtained from individuals.
Results: mtDNAcn was significantly correlated to peak intensity of deployment-related exposures ($r=0.47$, 95%CI=0.02 to 0.76, $p=0.04$). Veterans in HIGH exposure group had significantly higher mtDNAcn than Veterans in LOW exposure group ($t=2.51$, $p=0.02$). There was no significant difference in smoking history between HIGH and LOW exposure groups.
Conclusions: In respect to increased mtDNAcn in HIGH exposed Veterans, deployment-related exposures may be associated with mitochondrial dysfunction. Future studies should consider additional parameters of mitochondrial function as well as an exercise challenge to better understand the effects of deployment-related exposures.

Alendronate Treatment Elicits a Reduction in Mechanical Properties and the Density of Osteocyte Lacunae in Cortical Bone Tissue

Joseph Geissler
3rd Year PhD Student
Mentor: J. Christopher Fritton, PhD
Department: Orthopaedics
Poster N^{O}06

Bisphosphonates (BPs) are anti-resorptive drugs that decrease fragility fracture risk by decreasing individual osteoclast tunneling, and increasing and decreasing apoptosis of osteoclasts and osteocytes, respectively [1]. The emergence of a subset of atypical fractures with their defining characteristics (long-term use, low-energy, transverse fracture of the femoral mid-shaft cortex, bilaterality) has renewed interest in the effects of drugs on tissue-level mechanical properties and the mechanisms behind these fractures remains unknown [2]. In adult female beagle rib (n=12/group) we found that high-dose, long-term (3 years) alendronate (ALN) treatment reduces both resistance to failure (3-fold reduction in the fatigue-life) and stiffness (21% reduction) of uniform cortical tissue beams (1.5 x 0.5 x 10 mm) in a 4-point bending configuration (Bose Testbench, 2 Hz). Histological differences were also revealed. While not affecting the number of osteons, ALN treatment reduced the average size of individual osteons by 17%, and elicited a 20% reduction in cortical bone osteocyte lacunar density. Combined, these results suggest that tissue-level structural components normally contributing to healthy bone are altered by ALN treatment and contribute to reduced mechanical properties.
REFERENCES: [1] Cummings et al. JAMA 280, 2077-2082, 1998 [2] FDA Meetings of Advisory Committee for Reproductive Health Drugs and Drug Safety 2013 [3] Allen et al. Calcif Tissue Int 82, 354-360, 2008.
ACKNOWLEDGEMENTS: Linda Uko and Edek Williams provided technical assistance. Alendronate was provided at no cost by Merck. Funding was supported by NIH (C06 RR010601, AR062002, AR007581, AR047838, AR063351), NASA (NCC 9-58), Sigma Xi and the NJ Space Grant Consortium.

The Use of BMP-2 and Novel Polymers for Guided Bone Regeneration

Sangeeta Subramanian
4th Year PhD Student
Mentor: Kathryn Uhrich, PhD and J. Patrick O'Connor, PhD
Department: Orthopaedics
Poster N^{O}07

Bone morphogenetic protein-2 (BMP-2) has been shown to play an integral role in musculoskeletal development by inducing osteoblastic differentiation and is used clinically to promote bone regeneration. Adverse effects of BMP-2 such as excessive swelling have been documented in patients who underwent spinal fusion. NSAIDs have been proven to delay or prevent bony union in fractures by inhibiting cyclooxygenase-2. It has also been shown that BMP-2 treatment can overcome the negative effects of NSAIDs in bone repair. We have developed a novel material to improve bone healing by incorporating salicylic acid-based poly(anhydride ester) (SAPAE) with polycaprolactone (PCL) for use as a guided bone regeneration membrane. Two different SAPAE compositions (fast-degrading (FD) and slow-degrading (SD)) were fabricated then dissolved with PCL and electrospun into thin, malleable mats which were tested in vivo in a rat segmental defect. BMP-2 was delivered via a collagen sponge and the defect was wrapped with (i) nothing, (ii) PCL, (iii) FD-SAPAE, or (iii) SD-SAPAE. We hypothesized that using the PCL-SAPAE membrane to contain BMP-2 would enable bone regeneration while reducing ectopic bone formation compared to controls. There was no significant difference found in defect bone volume between groups. However, significantly less bone was found outside the defect space using the FD-SAPAE membrane when compared to the unwrapped control and the PCL membrane groups. The salicylic acid released from the FD-SAPAE membranes appears to limit ectopic bone formation while allowing bone to grow within the defect space, confirming the hypothesis.

The Effects of Macrophage and Osteoclast Depletion in Bone Fracture Repair

Hsuan-Ni Lin
5th Year PhD Student
Mentor: Patrick O'Connor, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}08

This study investigated the effects of macrophage and osteoclast depletion in fracture healing. Macrophages and osteoclasts are major cell types of the inflammatory stage and bone remodeling stage of fracture healing, respectively. Growth factors produced by macrophages promote mesenchymal cell recruitment, osteogenesis, and chondrogenesis. Osteoclasts regulate bone resorption in normal physiology and fracture healing. We treated wild-type ICR mice with clodronate-liposome (CLD-lip) to deplete macrophages, osteoclasts, and other monocyte derivatives. CLD-lip treatment reduced macrophage and osteoclast number to 30% of normal levels in fracture calluses and 10% in spleens. Fracture healing was impaired in CLD-lip treated mice compared to phosphate buffered saline-liposome (PBS-lip, control) treated mice. Endochondral ossification was abnormal in CLD-lip treated mice with evidence of delayed cartilage and bone resorption. Arachidonic acid (AA) metabolites are important regulators of the inflammatory response and fracture healing. Cyclooxygenase and 5-lipoxygenase are two major AA pathway enzymes that catalyze the production of prostaglandins and leukotrienes from AA, respectively. Macrophages and osteoclasts also produce AA pathway enzymes. We are particularly interested in the role of cyclooxygenase-2 (COX-2) produced by macrophages and osteoclasts in fracture repair. We knocked out COX-2 in macrophages and osteoclasts using floxed COX-2 mice and a Lyz2-Cre transgene. We found abnormal endochondral ossification and delayed fracture healing in COX-2(flox/flox);Lyz2Cre mice with unresorbed cartilage and disorganized new bone formation at day 21 compared to COX-2(flox/flox) mice. However, fracture impairment was less severe compared to the CLD-lip treated mice. The results suggest that macrophages and osteoclasts regulate fracture healing through a COX-2 mediated mechanism.

The SWI/SNF chromatin remodeling complex and control of osteoblast/adipocyte lineage determination

Kevin Nguyen
6th Year PhD Student
Mentor: Elizabeth Moran, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}09

The chromatin remodeling complex SWI/SNF, with its core ATPase BRG1, is required for tissue-specific gene expression. A subset of SWI/SNF, powered by the alternative ATPase BRM, generally has auxiliary effects. In osteoblast precursors, BRM-SWI/SNF represses premature expression of tissue-specific genes, whose activation is BRG1-dependent and BRM-independent. The role of BRM-SWI/SNF in other lineages that derive from bone marrow stromal cells (BMSCs) is largely unknown, but its role in adipocyte differentiation is of particular interest because an improper balance of adipocyte versus osteoblast differentiation underlies certain skeletal pathologies. This question was addressed by generating stable knockdown of BRM or BRG1 in 3T3-L1 preadipocytes. In contrast to their opposing roles in osteoblasts, BRM and BRG1 are both required for adipogenesis. Depletion of either ATPase has a similar effect in inhibiting adipogenesis and expression of key early and late adipocyte markers, whose promoters they co-target. The implication that BRM-SWI/SNF is a significant effector of lineage choice between osteoblasts and adipocytes was probed further by generating BRM deficiency in the multipotent mesenchymal stem cell model C3H10T1/2 cells. Depletion of BRM in these cells simultaneously enhances differentiation along the osteoblast pathway and impedes differentiation along the adipocyte pathway. The opposing role of BRM-SWI/SNF in osteoblast versus adipocyte differentiation suggests a future possibility of targeting BRM to promote bone regeneration. In support of this concept, examination of BMSCs from BRM-null mice shows that this cell population is relatively enriched for osteoblast precursors and impoverished for adipogenic potential. The enriched pool of osteoblast precursors is apparently an unused reservoir during normal development, as the BRM-null mice do not show aberrant bone overgrowth.

The apoptotic cell recognition receptors, Tyro3, Axl and Mer, show distinct patterns of ligand-inducible and ligand independent receptor activation

Wen-I Tsou
7th Year PhD Student
Mentor: Sergei V. Kotenko, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}10

Proper recognition and removal of apoptotic cells by phagocytes lead to the active suppression of inflammatory responses and the induction of tolerance. Tyro3, Axl and Mer (TAM) receptor tyrosine kinases recognize apoptotic cells through their ligands, Protein S (ProS) and Growth-Arrest-Specific Gene 6 (GAS6), which in turn interact with phosphatidylserine (PS) exposed on the surface of apoptotic cells. Although TAMs share significant similarity, very little is known about the specificity of interaction between TAMs and their ligands, in the context of apoptotic cells, and about downstream signaling cascades triggered through TAMs. To study ligand-receptor interaction, we generated a series of reporter cell lines expressing chimeric TAM receptors that allowed us to demonstrate that each TAM has a unique pattern of interaction with GAS6 and ProS, which is also differentially affected by the presence of apoptotic cells. We also developed another set of chimeric receptors that triggered ligand-independent activation of the TAM intracellular domains, leading to distinct patterns of phosphorylated signaling molecules, as well as different levels of cytokine induction. Overall, these studies suggest that despite their similarity, Tyro3, Axl and Mer are functionally unique in the recognition of apoptotic cells, activation of downstream signaling cascades and gene expression, and therefore, contribute differently to cell survival and the induction of tolerance during tumorigenesis.

Role of Interferon-λ in Intestinal Immunity to Virus Infection

Jian-Da Lin
4th Year PhD Student
Mentor: Sergei V. Kotenko, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}11

Type I interferons (IFNs) and type III IFNs exert their antiviral activities in the host response to viral infection. Type I IFNs signal through the Jak-STAT (Janus kinases–Signal Transducers and Activators of Transcription) pathway shared by type III IFNs. The type III IFN receptor-ligand system contributes to antiviral or other defenses by a mechanism similar to, but independent of, type I IFNs. Although type I IFNs have been well characterized and appreciated for their complex regulatory roles on immune cells, immune functions of type III IFNs, IFN-λs, are largely unknown in host immunity. In an ongoing study, we aim to investigate immunological roles of IFN-λs in intestinal immunity to rotavirus (RV) infection. Our preliminary data indicate the increased RV levels in stools of IFN-λ R1 knockout (KO) suckling mice at day 3 post infection. Furthermore, RV infection leads to the increased levels of MHC class II antigen expression in CD23+ B cells in mesenteric lymph node (MLN) and Peyer's patches (PP) of adult WT mice. These elevations of MHC class II antigen expression are not observed in B cells of IFN-λ R1 KO adult mice in response to either murine EW RV or Rhesus rotavirus (RRV) infection. These results suggest that in addition to providing innate antiviral protection in GI tract, IFN-λs play roles in intestinal immunity in response to RV infection by regulating B cell functions. In future, we will investigate mechanisms by which IFN-λs influence the intestinal B cell responses and the interplay between intestinal epithelial cells and B cells in response to RV infection with the use of conditional IFN-λ R1 KO mice.

Interferon (IFN) IFN-λ4, new addition to the type III IFN family

Satya Singh
3rd Year PhD Student
Mentor: Sergei V. Kotenko, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}12

Interferons (IFNs) are proteins secreted from virus-infected cells with antiviral activity. IFNs are pleotropic cytokines with antiviral, antiproliferative and immunomodulatory activities. Most cell types are capable of producing interferons (IFNs) in response to viral infections. IFN stimulation can execute direct antiviral effect such as degradation of viral genome and proteins via upregulating antriviral genes. Indirect effect of IFNs is produced by upregulation of genes associated with MHC class I expression. A novel cytokine, IFN-λ4 was recently added to the type III IFN family, but its receptor complex was not characterized. We demonstrate that participation of canonical IFN-λ receptor subunits, IFNLR1 and IL10R2 is necessary for the activation of the Jak-STAT signaling pathway in response to IFN-λ4, and likely for the induction of antiviral activity. Therefore, IFN-λ4 triggers activation of the transcriptional factor known as ISGF3 (Interferon Stimulated Gene Factor) that re-localizes to the nucleus and interacts with ISRE (Interferon Stimulated Response Elements), thereby promoting the transcription of IFN-stimulated genes (ISGs), including those involved with MHC Class I antigen presentation. To study the correlation between STAT activation and MHC class I upregulation in response to IFN-λ4, MHC class I expression in human retinal pigment epithelial cells (ARPE19) was analyzed by flow cytometry. Western blot analysis was used to qualitatively evaluate STAT1 activation. While expression of MHC class I appeared to be upregulated for over a time course of 72 hours, STAT activation was transient, suggesting that IFN-λ4 has unique biological properties amongst type III IFNs.

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

Ganapathy Sriram
6th Year PhD Student
Mentor: Raymond B. Birge, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}13

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, concommittant with Abl activation, upon induction of the EGFR signaling axis by EGF in MDA-MB-468 human breast cancer cells. Mutation of the Abl bindng 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.

Crk and Abi1 regulate Abl tyrosine kinase activity by a binary molecular switch mechanism

Sushil Kumar
3rd Year PhD Student
Mentor: Raymond Birge, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}14

Abl tyrosine kinase is an important mediator of various cellular processes that range from cell invasion, proliferation and motility to apoptosis. Constitutive activation of Abl tyrosine kinase leads to aberrant cellular proliferation and decreased apoptosis, which is seen in various cancer types including chronic myeloid leukemia. Here, we describe a novel regulatory mechanism of Abl kinase activity by a binary molecular switch involving Crk and Abi1 adapter proteins. Using a combination of Abi1-/-mouse embryonic cells, glioblastoma cells lines and in vitro kinase assays using recombinant proteins, we show that the SH3 domains of Crk and Abi1 compete for binding to a proline rich domain on Abl tyrosine kinase. Despite the fact that Crk and Abi1 compete for a binding site on Abl, they do so with different itineraries, where Crk is a positive regulator and Abi1 is a negative regulator of Abl tyrosine kinase activity. In such a binary regulation, we further show that Crk mediated transactivation of Abl kinase is enhanced by loss of Abi1 in glioblastoma cell lines, thus suggesting a putative tumor suppressor role for Abi1 in glioblastoma that remains to be tested. Together these data support a novel regulatory mechanism of cellular tyrosine kinases via competition of SH3 modular domains of adapter proteins.

TAMpering with Efferocytosis in Cancer

KhanhQuynh Nguyen
7th Year PhD Student
Mentor: Raymond Birge, PhD
Department: Biochemistry & Molecular Biology
Poster N^{O}15

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 (MΦ), 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 MΦ and DCs in tumor phagocytosis, there is a great need to understand the role of epithelial cancer cells as phagocytes since they have been shown to overexpress multiple phagocytic receptors. Among those, TAM receptor tyrosine kinases consisting Tyro-3, Axl, and Mer are often associated with highly invasive cancers and poor patient survival. In addition, TAM are knowingly required for apoptotic cell (AC) clearance by professional phagocytes, and actively involved in immune suppression. In the present study, our data revealed that epithelial cells had variable efferocytic capacity and most invasive breast cancer cells MDA-MB231 overexpressed the highest level of TAM and had greater phagocytic capacity. Moreover, transient or stable expression of Mer 21, MCF-1A, or Hela cells effectively enhanced efferocytosis. To further study Mer directed efferocytosis in cancer cells, Mer shRNA or inhibitors in form of TAM soluble receptor traps were employed. While Mer knocking-down only reduced 15% of phagocytosis in MDA-MB-231, TAM soluble receptor traps could inhibit efferocytosis in MDA-MB-231 and MCF-10A-Mer up to 40%. These data collectively identify TAM, especially Mer, as a significant link between cancer and efferocytosis, a potentially new and unrecognized oncogenic event that needs to be further explored.

IRF5 is a novel regulator of CXCL13 expression in breast cancer that increases B and T cell trafficking to the tumor

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Poster N^{O}16

In recent years, attention has turned to the role of non-neoplastic cells in the tumor microenvironment, including stromal cells and infiltrating leukocytes, whose presence may influence an individual patient's long-term outcome. Clinical studies using prognostic and predictive signatures have shown that the strength of the immune signal emanating from whole tumor gene expression profiles reflects the level of immune infiltration - a high immune signal has been linked with improved patient outcome. In this study, we examined cytokine/chemokine expression profiles between breast cancer cells lacking or expressing interferon regulatory factor 5 (IRF5) and determined whether loss of IRF5 alters immune cell trafficking to the tumor.

RNA was isolated from 3D cultures of MDA-MB-231 cells lacking or expressing IRF5 and cytokine/chemokine expression analyzed by focused array. Protein expression was examined in formalin-fixed paraffin-embedded (FFPE) archival breast tissue specimens. Migration assays were used to assess trafficking of immortalized B cells, monocytes and T cells, as well as primary immune cell subsets, to tumor-conditioned media (TCM) from IRF5-positive and -negative MDA-MB-231 cells.

Expression of a number of cytokines/chemokines was found to be dysregulated between IRF5-positive and -negative MDA-MB-231 cells grown in 3D culture. CXCL13 was identified as a direct target of IRF5. Loss of either IRF5 or CXCL13 expression resulted in significant decreases in both B and T cell trafficking to TCM. Expression of these two proteins was found to positively correlate in a large number of primary FFPE breast tumor tissues examined.

Loss of IRF5 expression in human breast cancer cells not only predisposes them to undergo metastasis, but also re-programs the tumor microenvironment towards immune evasion. Re-expression of IRF5 in breast cancer cells otherwise lacking expression makes the tumor more immunogenic through IRF5's ability to directly upregulate CXCL13 expression. Upregulation of CXCL13 expression results in enhanced B and T cell trafficking to the tumor. Results from this study support that IRF5 directly regulates a network of genes that shapes a tumor immune response and may, in combination with CXCL13, serve as a novel prognostic marker for immunotherapy response.

Novel Mechanism of Negative Regulation of 1,25-Dihydroxyvitamin D3 Induced 24(OH)ase Transcription: Epigenetic Modification Involving Crosstalk Between Protein Arginine Methyltransferase 5 and the SWI/SNF Complex

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Poster N^{O}17

The mammalian SWI/SNF chromatin remodeling complex facilitates gene transcription by remodeling chromatin using the energy of ATP hydrolysis. Each SWI/SNF contains two homologous ATPases, BRG-1 and hBRM. Recent studies have indicated an interplay between histone modifying enzymes and chromatin remodeling factors. Among the histone modifying enzymes are the protein arginine methyltransferases (PRMTs) which have been implicated in transcriptional activation or repression. Little is known however about the role of SWI/SNF and PRMTs in vitamin D receptor (VDR) mediated transcription. Using SW13 and C33A cells, which are deficient in BRM and BRG-1, we found that 1,25(OH)2D3 induction of 24(OH)ase transcription is markedly reduced (6 – 10 fold), suggesting that the SWI/SNF complex is an important component of VDR mediated transcription. Activation of transcription was restored in these cells preferentially by BRG-1. In UMR osteoblastic cells the N-terminal region of BRG-1 (BRG-1-N), that acts as a dominant negative inhibitor, inhibits 1,25(OH)2D3 induction of 24(OH)ase expression 2 fold as indicated by Western blot analysis. Mutant BRG-1 also inhibits C/EBP□□enhancement of VDR mediated 24(OH)ase transcription and expression. BRG-1-N at the concentrations used had no effect on basal promoter activity. Immunoprecipitation assays indicate that BRG-1 associates with C/EBP□. Chromatin immunoprecipitation assays show that C/EBP□ and BRG-1 bind simultaneously to the 24(OH)ase promoter. BRG-1 enhancement of VDR mediated transcription is not observed using a 24(OH)ase promoter construct with the C/EBP site mutated. Here we further show that PRMT5, a type II PRMT that dimethylates histone 3 at arginine 8 (H3R8) and histone 4 at arginine 3 (H4R3) inhibits BRG-1 and C/EBP□ enhancement of 1,25(OH)2D3 induced 24(OH)ase transcription. In the presence of PRMT5 (100ng) the 3 fold enhancement of VDR mediated 24(OH)ase transcription observed in the presence of BRG-1 and the 5 fold enhancement in the presence of both BRG-1 and C/EBPβ (100ng each) are inhibited ($p<0.05$ transcriptional activation in the presence or absence of PRMT5). Using a catalytically inactive PRMT5 (methylation of H3 and H4 is lost) negative regulation of 24(OH)ase transcription is not observed. ChIP assays show that PRMT5 associates with BRG-1 at the C/EBP site. These studies define epigenetic events linked to a novel mechanism of negative regulation of VDR mediated transcription.

Post-Transcriptional Regulation of COX-2 in Lung Cancer Cells

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Poster N^{O}18

The oxidative conversion of arachidonic acid to prostaglandin H2 is carried out by a set of two enzymes termed cyclooxygenases, abbreviated as COX. COX-1 is constitutively expressed in normal tissues, while COX-2 is transiently induced from external stimuli, such as pro-inflammatory cytokines. COX-2 is also overexpressed in numerous cancers. We show that COX-2 protein expression is constitutive in a lung cancer cell line, A549, but is not expressed in a normal bronchial cell line, Beas2B. The 3'UTR of COX-2 serves as a region by which COX-2 expression can be tightly regulated. One such mechanism of post-transcriptional regulation of COX-2 is mediated through microRNAs. Data show that transient transfection of miR-146a expression in A549 cells specifically represses COX-2 protein. This action also suggests that COX-2 enzymatic function is also negatively impacted by miR-146a. 3'UTR reporter assay data provide evidence for a direct relationship of COX-2 and miR-146a. We speculate that many mechanisms act in concert to regulate COX-2 gene expression.

Mechanism of Macrophage Phenotype Regulation by MicroRNA

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Poster N^{O}19

Macrophages are versatile immune cells that play many roles including regulation of inflammation and wound healing. Persistently activated macrophages are a major factor causing chronic inflammation. Macrophage activation is achieved by cytokines and other stimulants that drive them towards either inflammatory (M1) or anti-inflammatory (M2) phenotypes. Switching M1 macrophages to M2 macrophages may alleviate inflammation and lead to tissue repair. Our labs aim to investigate the molecular mechanisms underlying the phenotypic switch of macrophages from an M1 to M2-like phenotype when murine macrophages are stimulated with LPS and NECA. Accumulating evidence shows that macrophage programming and phenotypic switching are regulated by different signal molecules. Recent studies showed that miR-155-regulated inflammatory cytokine production in tumor associated macrophages (He, Xu et al. 2009). We aim to investigate miRNAs that might be one of the key molecules controlling M1 to M2-like macrophage phenotypic switch when murine macrophages are stimulated with LPS/NECA.

Post-transcriptional Regulation of PLCβ2 in LPS and adenosine-mediated activation of macrophages

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Poster N^{O}20

Macrophages can be classically activated (M1) or alternatively activated (M2) depending upon the stimulating factor/s and the cytokines and growth factors that they produce on stimulation with the particular factor. M1 activation is induced by exposure to LPS with or without IFN□□and leads to the development of a pro-inflammatory phenotype necessary for the clearance of foreign pathogens and damaged tissue. Conversely, M2 activation promotes macrophage polarization into an anti-inflammatory phenotype in order to effect tissue repair and remodeling as well as the resolution of the inflammatory response. M2 macrophage activation is induced by a variety of signals, and studies from our lab have unmasked a novel 'switching' of macrophages from a 'classical' to an 'alternatively' activated phenotype that we have termed "M2d". This switch is induced by signaling through adenosine A2A receptors (A2ARs). We have observed that LPS rapidly and specifically up-regulates expression of A2ARs, and also suppresses phospholipase C-□2 (PLC-□2) expression in macrophages at the post transcriptional level by destabilizing its mRNA. This suppression was shown to play a role in modulating the A2A receptor signaling underpinning this phenotypic 'switch'. The mechanism of destabilization of PLC-□2 mRNA remains unknown. Bioinformatic analysis of the 3'UTR of PLC-□2 mRNA has indicated a direct and conserved binding site for microRNA 466L which overlaps with a binding site for RNA binding proteins like TTP and HuR which are known to be involved in mRNA stability. Luciferase reporter experiments using the PLCβ2 3'UTR construct showed that the LPS mediated PLCβ2 mRNA down regulation is takes place through the 3'UTR of PLCβ2 mRNA. Although, there is a strong induction of TTP upon LPS treatment of macrophages, site-directed mutagenesis of the AU rich regions (where TTP is known to bind and act) in the 3'UTR did not show any effect on the LPS mediated suppression of reporter expression indicating that they might not be involved in the PLCβ2 mRNA destabilization upon LPS treatment of macrophages. Also, PLCβ2 expression in mRNA from LPS treated macrophages of TTP KO mice showed LPS mediated PLCβ2 down regulation just as it did in WT macrophages further confirming that TTP is not involved the PLCβ2 mRNA destabilization. Systematic deletions of the regions of the 3'UTR to determine the cis-element responsible for LPS mediated PLCβ2 mRNA destabilization is currently underway. Moreover, microRNA profiling of LPS and adenosine treated macrophages by microarray revealed distinct subsets of microRNAs that are regulated by LPS alone and by LPS and A2AR signaling. We are also investigating the influence of the candidate miRNAs on the stability of PLC-□2 mRNA in macrophages as well as on adenosine-mediated alternative macrophage activation. These studies involve both knockdown and overexpression of candidate miRNAs coupled with subsequent analysis of the expression of several M1 (e.g. TNF-□, iNOS) and M2d markers (e.g VEGF, IL-10 and A2ARs) as well as canonical markers of M1 and M2 activation. Over expression of miRNA466L and miRNA155 in murine peritoneal macrophages with the use of mimics showed decreased expression of PLCb2 mRNA relative to the negative control (mock) mimic treated macrophages suggesting the roles of these microRNAs in the stability of PLC-□2 mRNA. In terms of the cytokine markers of M2d activation upon over expression of miR466L and miR155, we looked at TNF-α for M1 activation, and VEGF for M2 activation. While the expression of TNF alpha (in LPS treated macrophages) seemed unaffected by the over expression of miR155 and miR466L, the expression of VEGF (in LPS and adenosine receptor agonist NECA treated macrophages) was lower than in the negative control (mock) mimic treated macrophages. Investigations on the effect of inhibition of these microRNAs on PLC-□2 mRNA levels as well as on the expression of M1 and M2 cytokine markers (TNF-α and VEGF respectively) are currently underway.

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Hematopoietic Id Ablation Triggers Endomyocardial Fibrotic and Vascular Defects Within the Murine Heart

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Poster N^{O}21

We previously reported that Id (inhibitor of DNA binding protein) deficient mice with endothelial and hematopoietic (Tie2Cre) ablation (Id conditional double knockout mice, Id cKO) survive into adulthood and develop dilated fibrotic cardiomyopathy (DCM) and multiple hematopoietic defects. The Id cKO hearts exhibit perivascular fibrosis and interstitial fibrosis within the endomyocardium along with disruption of the endocardial lining. To determine if hematopoietic Id ablation contributes to cardiac pathology, we conducted a series of bone marrow transplantation studies to control the Id status of the hematopoietic system. We found that Id cKO bone marrow transplantation phenocopies the Id cKO hearts 4 months post-transplantation with a corresponding decrease in ejection fraction in 8 out of 13 WT recipients (Id cKO/WT BMTs). Real-time PCR analysis of Id cKO/WT BMT hearts reveals a 2.1-fold increase ($p<0.05$) in the expression of thrombospondin-1 (TSP1), which is normally repressed by Id1 compared to WT/WT BMT controls. Connective tissue growth factor (CTGF) - a downstream target of the TSP1/TGFbeta1/Smad pathway - exhibits a 2.3-fold ($p<0.05$) increase in mRNA expression Similarly, collagen type III alpha 1 (Col3a1) - a collagen marker typically upregulated in the context of DCM - is upregulated 2.09-fold ($p=0.05$). No significant changes in Id1 expression was observed although there was a trend toward higher levels in Id cKO/WT BMTs. No significant changes in tumor necrosis factor receptor associated factor 6 (TRAF6) - a member of the non-Smad/CTGF pathway - expression was observed. Insulin growth factor binding protein 3 (IGFbp3), a factor that directly activates the Smad pathway independent of TGFbeta1 signaling, was upregulated 6.45-fold ($p<0.05$). Treatment of HUVEC cells with Id cKO serum on matrigel shows a 30.7% decrease in the total number of tubes. The results highlight a novel pathway whereby loss of distal Id from bone marrow cells triggers upregulation of TSP1 and local expression of Id in the heart is insufficient to prevent dysregulation of TSP1. The findings suggest that Id ablation in hematopoietic cells triggers dysregulation of vascular and fibrotic pathways in the hearts of Id cKO/WT BMTs, possibly mediated by the TSP1/CTGF pathway.

Dominant effect of muscular dystrophy ESCs in WT mice

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Poster N^{O}22

We previously created chimeric mice by injecting wild-type (WT) mouse embryonic stem (ES) cells and induced pluripotent stem cells into mdx (dystrophin-negative) blastocysts, which are predisposed to develop symptoms of Duchenne muscular dystrophy (DMD). Interestingly, we determined that a low percentage of ES cells was sufficient to supply dystrophin to the heart and skeletal muscles, producing a significant amelioration of disease.
Recently, we generated mosaic mice by injecting mdx ES cells into WT blastocysts (termed 'reverse' chimeras). With low levels of ES cell incorporation (10-30%), the mdx/WT chimeric heart acquired dystrophic features, specifically in terms of intracellular calcium responses to mechanical stress, well before normal onset would occur in the mdx heart. While the ES-derived mdx cardiac myocytes behaved like typical mdx cardiac myocytes, surprisingly the embryo-derived WT cardiac myocytes also behaved as mdx cardiac myocytes. In addition to the effects on the heart, we observed that at a higher degree of chimerism (30-50%), specific skeletal muscles like the pectoralis and diaphragm, but not the quadriceps or soleus, showed histological features of muscular dystrophy. Affected muscles displayed both a non-uniform expression of dystrophin and compromised utrophin upregulation in fibers with negligible dystrophin. Preliminary functional studies revealed that the EDL muscles, but not the soleus muscles, are sensitive to mdx ES cell chimerism, as the EDL twitch, tetanic forces, and shortening velocities were attenuated. At similar levels of chimerism, mature adipocytes showed an upregulation of muscle and cardiac markers, and evaluation of related changes in secreted hypertrophic factors such as Wnt5a and follistatin-like proteins is underway. Together, our findings suggest that the ES-derived mdx compartment dictates the overall phenotype in the heart, certain skeletal muscles, and the fat of these 'reverse' chimeras.
As mosaicism is a common feature in DMD carriers (dystrophin +/-) and in Becker muscular dystrophy, this unsuspected dominant, muscle specific function of the mdx ES-derived cells merits further studies.

Cyclophilin D Deficiency Attenuates Ca2+ Waves During Mitochondrial Depolarization in Mouse Cardiomyocytes

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Poster N^{O}23

Recent studies have indicated that mitochondrial Ca flux plays a significant role in modulating micro-domain Ca2+ levels, near the ryanodine receptors, and spontaneous Ca wave (CaW) behaviors in isolated cardiomyocytes. In the present study, we have used a genetic mouse model which lacks cyclophilin D (CypD KO), a necessary component for the opening of the mitochondrial permeability transition pore (mPTP), to further assess the hypothesis that mPTP plays a central role in the regulation of CaWs during mitochondrial depolarization. Ventricular myocytes were isolated from wild type (WT) and CypD KO mice. Mitochondrial calcein release, as estimated from the fluorescence decrease, was used as the index of mPTP opening. Cytosolic Ca2+ was imaged using Fluo-4-AM. Spontaneous CaWs were induced in the presence of high external Ca2+ (4 mM). The protonophore carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP) was used to depolarize mitochondrial membrane potential ($\Delta\Psi m$). WT myocytes showed a significant decrease (61%) in calcein fluorescence in the presence of FCCP (10 μM), compared to CypD KO myocytes (28%; $p < 0.05$, independent samples t-test). These results indicate less opening of mPTP in response to $\Delta\Psi m$ depolarization in CypD KO myocytes. Consistent with these results, FCCP (50-500 nM) significantly increased the frequency of CaWs in WT myocytes, however no significant increase was observed in CypD KO myocytes. For example, 50 nM FCCP significantly increased the frequency of CaWs from 12.9 ± 2.2 to 32.2 ± 5.3 min-1 in WT myocytes ($p < 0.05$, paired t-test), while no significant increase was seen in CypD KO myocytes (26.6 ± 4.3 to 24.4 ± 4.4 min-1). The effects of FCCP on Ca2+ waves in WT mice were attenuated by the mPTP blocker cyclosporin A. Finally, FCCP increased CaW frequency when cellular ATP consumption was inhibited by oligomycin A (1 μM), an ATP synthase inhibitor, thus supporting the notion that mitochondrial Ca fluxes play a vital role in the regulation of CaWs. In conclusion, cyclophilin D deficiency attenuates Ca2+ waves during mitochondrial depolarization induced by FCCP, suggesting that mitochondrial Ca release via mPTP opening plays an important role in the regulation of intracellular CaWs and arrhythmogenesis.

Unique exonic RNA polymerase II pausing in the Hif1a gene is associated with alternative splicing

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Poster N^{O}24

Unique exonic RNA polymerase II pausing in the Hif1a gene is associated with alternative splicing
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Hypoxia-inducible factor 1α (Hif-1α) is the primary transcription factor that promptly increases upon exposure of the cells to hypoxia. It is mainly regulated by posttranscriptional and posttranslational mechanisms involving miR-199a and prolyl hydroxylase, respectively. In addition, the discovery of multiple Hif-1α isoforms suggests that alternative splicing may play a role in regulating its activity, although little is known about this in the heart. We, thus, hypothesized, that in addition to the known mechanisms, Hif-1α is activated by an alternative splicing step, a process that is tightly coupled to transcription. To test this, we treated myocytes with antimiR-199a, which robustly induces Hif-1α expression, and analyzed its effects on gene transcription using RNA polymerase II Chromatin immunoprecipitation-deep sequencing. The analysis revealed that antimiR-199a induced 1) the upregulation of genes that are predominantly Hif-1α targets including Pdk1, Glut3, Vegfa, Bnip3, Ndrg1, and Gys1, in addition to Pfkm, G0s2 and Sdpr, which are a subject of an independent study, and 2) a unique, very precise RNA pol II pausing (accumulation of pol II at a very precise sites with a density >2x of its flanking sequence) within the exons of only the Hif-1α gene, while RNA pol II at the transcription start site, and within other regions of the gene body, was similar to the control myocytes. In particular, all exons except for exons 1, 10, and 11, exhibited some degree of pausing, where exon 15 had a pol II pausing peak that was 32 fold higher than the flanking pol II density. Deceleration of transcriptional elongation has been associated with RNA splicing, however, these very precise exonic pausing peaks are quite unique. Using RNA pol II chromatin Chromatin immunoprecipitation- quantitative PCR (ChIP-qPCR), were able to confirm that during normoxia, Hif-1α was abundant as determined by primers specific for exons 10, 11, and 12, which increased by ~2 folds with miR-199a treatment. On the other hand, exon 15, which encodes part of the transactivation domain, was relatively lower during normoxia but increased 300x with antimiR-199a treatment. The result suggests that alternative splicing of exon 15 is a critical regulatory step required for the transcriptional activity of Hif-1α associated with the increase in its protein. The RNA pol II peak within exon 15 was associated with a 67x increase in H3K27-trimethyl, which is a hallmark of paused genes. Thus, the data suggest a unique mode of Hif-1α regulation via differential exon splicing of its transactivation domain region.

The reserve respiratory capacity in cardiac myocytes is regulated by metabolic substrates, hypoxia, and miR-199a

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Poster N^{O}25

Preconditioning of the heart to ischemia is one of the most effective measures in reducing the damage inflicted by ischemia. An aspect of ischemia preconditioning that has not been investigated is its effect on fatty acid and glucose metabolism; in particular, its effect on the reserve respiratory capacity (RRC) of the myocytes. Under normal conditions the cell runs on a fraction of its bioenergetic capacity, and the difference between its basal and maximum respiratory capacity is the RRC. A reduction in RRC has been associated with cell death and neurodegenerative disease; however, we have no knowledge regarding RRC regulation in cardiac myocytes during health or disease. We hypothesized that RRC is regulated in the myocytes by hypoxia and regulators of metabolism. To test this, we monitored the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) in real time in neonatal cardiac myocytes under different conditions, and after incubating them with various reagents to assess mitochondrial function. Our data show that myocytes, under normoxic conditions, had a 200% of basal RRC when palmitate was the source of energy. In contrast, there was no RRC in the presence of glucose, which induced a surge in glycolysis. After 24 h incubation in < 1% O2, RCC is completely obliterated in the presence of either substrate; basal OCR is reduced by ~30%; glucose increases glycolysis to levels equivalent to normal cells, however, it reduces OCR to ~10% of basal levels; while palmitate is more effective in sustaining OCR during the reoxygenation period. Since miR-199a, Hif-1a and their targets play major roles in hypoxia preconditioning and metabolism, we tested their roles in regulating RCC. Our results show that miR-199a reduces glucose oxidation while increasing glycolysis, glucose-, and fatty acid-dependent RRC. These effects are partly mediated by Pdk1, Glut3, and G0s2, which are transcriptionally-induced by antimiR-199a via Hif-1a in myocytes. In conclusion, our study shows that the RRC in myocytes favors fatty acids and is exhausted by hypoxia. Furthermore, inhibiting miR-199a and upregulating Hif-1a has a positive effect on RRC, which potentially mediates the hypoxia preconditioning effects of these molecules.

S-nitrosylation of thioredoxin 1 at Cys73 promotes transnitrosylation and cell survival during glucose deprivation

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Poster N^{O}26

Thioredoxin-1 (Trx1), a key anti-oxidant protein, is cardioprotective during oxidative stress mainly through its activity as an oxidoreductase. Trx1 can be S-nitrosylated and, in turn, can trans-nitrosylate other proteins. However, the role of Trx1-dependent S-nitrosylation in cardiomyocytes is unknown. Here, we investigated the role of Trx1 mediated protein S-nitrosylation in the regulation of cardiomyocyte survival in response to stress. Using the biotin switch assay, we found that Trx1 is S-nitrosylated at Cys73. Overall protein S-nitrosylation in rat neonatal cardiomyocytes was increased in response to 4hrs of glucose deprivation (GD). Overexpression of wild-type Trx1 increased, whereas short-hairpin RNA-mediated knockdown of Trx1 (shTrx1) or overexpression of Trx1C73S decreased, total protein S-nitrosylation in response to GD. These results suggest that Trx1Cys73 plays a key role in the regulation of protein S-nitrosylation in cardiomyocytes during GD. After 24hrs of GD, overexpression of Trx1 increased cardiomyocyte survival while shTrx1 or overexpression of Trx1 C73S increased cell death. Autophagy is a pro-survival mechanism during GD. Therefore, we tested the effect of Trx1 on autophagy. After 4 hrs of GD, autophagy was stimulated by Trx1WT overexpression compared to control cells. However, overexpression of Trx1C73S decreased autophagy compared to overexpression of Lac Z in control cells. In addition, we observed that the redox activity of Trx1 was intact in the Trx1C73S mutant protein. These data suggests that S-nitrosylation of Trx1 at Cys73 is associated with an overall increase in protein S-nitrosylation in cardiomyocytes and promotes autophagy and cell survival during GD.

Bone Marrow is a Source of Nonhematopoietic Endometrial Stromal and Epithelial Compartment Cells in a Murine Model

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Poster N^{O}27

Human endometrium has the remarkable ability to regenerate all cellular compartments with every menstrual cycle; the cellular source(s) of which remain unknown. The objective of the current studies was to determine whether the bone marrow (BM) is a source of multiple endometrial cell types using a murine BM transplant model. BM cells were harvested from transgenic donor mice which ubiquitously express Green Fluorescent Protein (GFP) and injected into lethally irradiated, syngeneic female recipient mice. Recipients with successful hematopoietic reconstitution were sacrificed at 3, 5, 9 and 12 months post transplant and hysterectomy was performed. Numbers of GFP+, CD45+, and CD45- cells in the endometrial stromal and epithelial compartments were determined. In the stromal compartment, bone marrow-derived cells (BMDCs) were detectable as early as 3 months post transplant, and the BM remained a long term contributor of nonhematopoietic endometrial cells. Nonhematopoietic endometrial cells comprised 47.3-72.2% of total BMDCs in the stromal compartment at 12 months post transplant. In contrast, BMDCs were not detected in the glandular or luminal epithelial compartments until 12 months post transplant. These data demonstrate that the BM is a significant source of nonhematopoietic endometrial stromal compartment cells, and contributes to a much smaller extent to the epithelial compartments. That BM is a source of nonhematopoietic cells in the endometrial stromal and epithelial compartments provides a potential mechanism by which monthly regeneration of the endometrium may occur.

Cancer-Associated Fibroblasts and the Propagation of Genomic Instability

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Poster N^{O}28

The stromal fraction of the tumor microenvironment has recently gained much attention due to its involvement in tumor initiation, progression, metastasis, and recurrence. In many tumors, such stroma, composed mainly of Cancer-Associated Fibroblasts (CAFs), comprises as much as 50-70% of the tumor, making it a vital component of the tumor environment. In addition, new evidence has reportedly revealed a radioresistant nature of CAFs and an ability to rescue surrounding cancer cells from the killing effects of radiation. These observations suggest a supporting role of CAFs in therapy failure and an associated poor clinical outcome. To date, very little research has characterized the early stages of CAF generation and/or investigated the mechanisms underlying the ability of CAFs to withstand ionizing radiation and the resulting effects on surrounding cells. Such research is of immense importance in understanding the factors that impact radiotherapeutic responses.

Through initial characterization of the transition of normal fibroblasts to CAFs, we have identified enhanced genomic instability in normal human fibroblasts (AG1522) grown in a transwell insert co-culture model with MDA-MB-231 or MCF-7 breast cancer epithelial cells. Interestingly, this initial instability was absent after prolonged co-culture, suggesting an apparent adaptive response in AG1522. An additional explanation may include an induced cellular senescence and/or cell death, as suggested by the observed decrease in plating efficiency, which was inversely correlated with extended co-culture duration. Interestingly, the progeny of AG1522 cells co-cultured with MCF-7 cells also exhibited increased genomic instability following 22 population-doublings, with the genomic instability increasing with the duration of the parental co-culture. This finding highlights the potential involvement of CAFs in the perturbation of genomic stability within the tumor microenvironment, which may both promote increased aggressiveness in the primary cancer and lead to the emergence of secondary cancers. Lastly, CAFs have been suggested to protect/rescue neighboring cancer cells from ionizing radiation, which is consistent with our observation of enhanced potentially lethal damage repair (PLDR) in irradiated MDA cells co-cultured with AG1522 CAFs. This observation is critical when one considers the therapeutic use of fractionated radiotherapy and the increased therapy failure when CAFs are present within the tumor microenvironment.

Exploring the Role of Intercellular Communication in Response to Ionizing Radiation-Induced DNA Damage

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Poster N^{O}29

During most forms of cancer radiotherapy, only parts of the body are exposed to radiation at any one time. Traditionally, it has been assumed that biological effects would occur only in the irradiated cells. However, recent evidence indicates that the non-targeted (bystander) cells are also affected because they receive signals from irradiated cells. We hypothesized that the effects seen in these bystander cells is conveyed through signaling pathways involving phosphorylation. To test this hypothesis, we used mass spectrometry (LC-MS/MS) with SILAC labeling to identify phosphorylated proteins in directly-irradiated and bystander cells after isosurvival doses of α or γ-irradiation (10% survival at 80cGy and 400cGy, respectively). The results showed dramatically increased and decreased phosphorylation levels in many proteins. Signaling pathways involving these proteins were identified using Ingenuity software. Western blot analysis of phosphorylated annexin A2 in bystander cells verified the phosphorylation levels.

We focused on the mechanism of annexin A2 phosphorylation. A time course study of annexin A2 phosphorylation levels in the bystander cells suggested that this annexin A2 was actually from the directly irradiated cells, transported through exosomes. We tested this possibility and showed that cells incubated with medium from irradiated cells had increased phosphorylated annexin A2 levels relative to control. Further, we show that growth medium harvested from irradiated cells contains exosomes.

Reactivation of breast cancer micrometastases in the bone marrow

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Poster N^{O}30

Evidence suggests that breast cancer cells metastasize very early in the course of the disease. At the time of diagnosis more than a third of breast cancer patients with localized disease have micrometastases to the bone marrow, one of the primary sites of dissemination, and their presence represents an unfavorable prognosis. Although treatment of early localized breast cancer can be successful, the tumor can recur either locally or as distant metastases years or decades later. Such delayed recurrence is consistent with the concept of tumor dormancy. The mechanisms of dormancy and recurrence are poorly understood but evidence suggests a dependence on a close association with bone marrow stroma, which plays a dual role for micrometastatic breast cancer cells.

The bone marrow microenvironment provides a favorable niche for micrometastases to remain dormant for years and decades. We have demonstrated that FGF-2 can induce dormancy of well-differentiated breast cancer cells on fibronectin or on human stromal co-cultures in an in vitro model. Stromal cells export growth factors, which induce redifferentiation in the metastasized cancer cells and allow them to bind to structural proteins initiating signaling that support tumor dormancy.

Further, the bone marrow microenvironment plays a role in cancer recurrence. Approximately 50% of patients who have micrometastases develop recurrent disease, with most recurrences occurring in post-menopausal women. We hypothesized that stromal cells undergo senescence due to aging and/or post-menopausal estrogen deprivation and begin to secrete inflammatory cytokines that can stimulate dormant cancer cells to reawaken. In our model, by injuring murine stroma with hypoxia, oxidative stress and estrogen deprivation, we induced the secretion of the inflammatory cytokines IL-6, IL-8 and TGF-β. We also demonstrated that exogenous IL-6, IL-8 and TGF-β can reactivate dormant breast cancer cells in our in vitro model.

IL-12 regulated checkpoints in the CD8+ T Cell Response to Toxoplasma gondii

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Poster N^{O}31

We have previously shown that IL-12 is necessary for the differentiation of KLRG1+ IFN-γ producing CD8+ T effector cells during the immune response to Toxoplasma gondii. However, which key events during the activation, differentiation, and mobilization of CD8+ T effector cells are critically regulated by IL-12 have not been identified. By tracking the dynamics of endogenous and adoptively transferred T. gondii-reactive CD8+ T cells, we were able to identify critical IL-12 extrinsic and intrinsic effects on mature IFN-γ-producing T effector cells. IL-12 is critical for the early diversification of low frequency of T. gondii specific CD8+ T cells into KLRG1+ IFN-γ producing T effector cells, which occurs even prior to their initial proliferation. Unexpectedly, acquisition of KLRG1 is accompanied by an attenuation of CXCR3 expression and this down regulation is indirectly IL-12 dependent. We postulate that the CXCR3 reduction on these mature KLRG1+ CD8+ T effector cells allows for their outmigration from secondary lymphoid organs. Failure to suppress CXCR3 may result in retention of the KLRG1+ CD8+ T effector cells and thus result in the deficit of effector cells numbers observed at the site of T. gondii infection in IL-12p35-/- hosts. Our study reveals that IL-12 has major intrinsic and extrinsic effects on the early diversification of CTLs by promoting and attenuating expression of key CD8+ T lymphocyte genes required for their effector function and mobilization.

CCR2+ Inflammatory Monocytes Play an Essential Role in Defense Against Invasive Aspergillosis

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Poster N^{O}32

Aspergillus fumigatus is the etiological agent of over 90% of the invasive aspergillosis (IA) cases in immunocompromised individuals, and is considered the most commonly inhaled fungal pathogen. Innate and adaptive immune responses to A. fumigatus are central in preventing IA. Neutropenia is associated with IA development in humans and in experimental IA in mice. Thus neutrophils are considered as the primary innate cell type responsible for preventing IA. In this study, we explored whether inflammatory monocytes (Ly6C+CD11b+CCR2+) play a role in defense against IA. We employed a novel mouse strain where the CC chemokine receptor 2 (CCR2) promoter drives the expression of a simian diphteria toxin receptor (CCR2---DTR mice) where diphtheria toxin (DT) injection leads to the selective depletion of inflammatory monocytes (CCR2+Mo). CCR2---DTR and control mice were treated with DT before and after intra---tracheal A. fumigatus infection to maintain depletion of CCR2+Mo. DT---treated CCR2---DTR mice displayed 100% mortality in contrast to 100% survival in control animals. Extensive fungal growth was observed in the lungs of CCR2---DTR mice while control animals showed only the presence of ungerminated fungal spores demonstrating that mortality in CCR2---DTR mice is linked to IA development. Depletion of CCR2+Mo did not result in impaired neutrophil recruitment and flowcytometric examination of lung homogenates showed comparable numbers of neutrophils that infiltrated the lung in CCR2---DTR and control mice. To examine whether Mo and their derivatives directly kill A. fumigatus conidia, we examined conidial uptake and killing in vivo using fluorescent Aspergillus reporter (FLARE) conidia that harness fluorescence to trace conidial fate and measure Mo conidiacidal activity in the lung. We find that Mo and Mo---DC kill fungal spores in vivo with similar efficiency as neutrophils, suggesting that these cell types have conidiacidal activity. Our findings indicate that Mo and Mo---DCs contribute an essential host defense function against inhaled A. fumigatus conidia that is independent and separable from the essential role of neutrophils. Our findings indicate that Mo and their derivative Mo---DCs are required for defense against IA due to their dual role as orchestrators of innate and adaptive immunity against A. fumigatus spores.

A foil to itself: TLR2 signaling protects and damages host tissue during Mycobacterium tuberculosis infection

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Poster N^{O}33

Activation of Toll-like receptors (TLRs) is a critical first step in host control of Mycobacterium tuberculosis (Mtb). TLR2 warrants particular attention because TLR2 polymorphisms have been associated with increased susceptibility to tuberculosis. Utilizing a TLR2-deficient mouse (TLR2KO), we have demonstrated that TLR2 is a critical regulator of the granuloma, which ordinarily sequesters Mtb-infected macrophages and limits pulmonary immunopathology (McBride, A., Konowich J., and Salgame, P., 2013). These findings, coupled with recent publications illustrating a role for epithelial cells in host immunity against Mtb, led us to investigate the role of TLR2 in host defense against Mtb on hematopoietic and nonhematopoietic cells. We generated radiation bone marrow chimeric mice, which were lacking TLR2 in either in the hematopoietic (H-TLR2KO), nonhematopoietic (NH-TLR2KO) component, or both (TLR2KO) and infected these mice with aerosolized Erdman Mtb. Evaluation of these groups demonstrated that TLR2 signaling on the hematopoietic component is crucial for control of chronic murine Mtb infection. Loss of TLR2 in the hematopoietic component resulted in increased bacterial burden and inflammation, decreased accumulation of regulatory T cells, and disruption of the granuloma architecture, and therefore, a phenotype similar to, if not worse than, the TLR2KO. In contrast, loss of TLR2 on the nonhematopoietic component resulted in tighter containment of the pulmonary granulomatous inflammation resulting in decreased immunopathology, reduced dissemination of Mtb to extrapulmonary sites, and decreased cellular recruitment to the lung in comparison to Mtb-infected WT mice. These data illustrate a novel role for TLR2 on nonhematopoietic cells in Mtb-infection and a paradox within TLR2 signaling: TLR2 signaling on hematopoietic cells is protective to the host both by controlling infection and regulating inflammation while its role on nonhematopoietic cells is deleterious to the host and elicits inflammation induced immunopathology. These opposing effects of TLR2 provide a balanced response and are critical for proper maintenance of the granuloma during chronicity. Preservation of this delicate balance is key to maintaining host control of TB. A deeper understanding of TLR2's role in host mediated inflammation could lead to the development of cell-specific TLR2 inhibitors that limit inflammation-induced TB pathology and reduce the rate of reactivation of TB.

Characterization of Clinical Isolates of Mycobacterium tuberculosis : Host-pathogen Interactions

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Poster N^{O}34

Tuberculosis (TB) is primarily a pulmonary disease that is transmitted from person to person via aerosolized droplets containing Mycobacterium tuberculosis (Mtb). What makes this disease distinct is that exposure to Mtb can result in diverse clinical outcomes. With an aim to better understand host resistance and how bacterial factors might influence infection outcomes, we conducted a collaborative household contact study (HHC) in Vitoria, Brazil. Here, individuals were identified based on their exposure to Mtb aerosols from an "index case" of active pulmonary TB. The goal of this sub-study is to understand the role of strain variation in TB transmission and pathogenesis. As part of the initial analysis of the household contacts, 10 clinical isolates were collected from different index cases and grouped into high and low transmission phenotypes, with 5 in each group. Based on this categorization, we hypothesize that high transmission status of certain isolates might in fact be reflective of a highly virulent pattern of infection. Building further on this, we aim to distinguish the isolates based on their interactions with human immune cells. Through ongoing experiments we are examining post-infection variations in cytokine/chemokine profiles induced by the isolates. Using monocyte-derived macrophages we are also assessing intracellular survival kinetics of the strains. We also plan to further validate the mechanistic interactions of these pathogenic strains with the host by using murine models. These experiments will provide insights in drawing correlations between strain transmissibility and host resistance/susceptibility mechanisms. In the future, these results can be useful in finding correlates of risk and relapse among TB patients and also help in designing interventions to curtail further transmission within the population.

Induction of memory immunity to Mycobacterium tuberculosis does not depend on TLR9 signaling

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Poster N^{O}35

Recognition of Mycobacterium tuberculosis (Mtb) ligands by Toll like receptors (TLRs) present on antigen presenting cells (APCs) induces signaling cascades leading to the production of inflammatory cytokines, such as IL-12 and TNF-alpha and the generation of antigen specific adaptive immunity. In dendritic cells, TLR9 sequestered in endosomal compartments has been shown to produce IL-12 upon recognition of Mtb DNA. In vitro studies have demonstrated the insufficient production of IL-12 in dendritic cells lacking TLR9 signaling. In mouse models, the involvement of TLR9 in host resistance during acute Mtb infection has been shown to be minimal. However, the role of TLR9 towards the generation of memory immunity to Mtb has not been explored. We generated a memory immunity model in WT and TLR9-/- mice by immunizing with the ∆leucine ∆pantothenate double auxotroph of Mtb. Upon challenge with Mtb-Erdman, WT and TLR9-/- mice demonstrated similarly enhanced control of bacterial burden compared to their unimmunized counterparts. The granulomatous response, interferon-gamma production and the frequencies of different cell types recruited into the lungs were similar in the two genotypes. These findings suggest that induction of memory immunity to Mtb does not depend on TLR9 signaling.

Interactions of the Kaposi's sarcoma-associated herpesvirus (KSHV) Rta protein with RBP-Jk, the DNA binding component of the Notch pathway, define multiple classes of RBP-Jk target genes .

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Poster N^{O}36

KSHV is a DNA tumor virus implicated in Kaposi's sarcoma (KS), Primary effusion lymphoma (PEL) and Multicentric Castleman's disease (MCD). Reactivation of KSHV from latency is essential for dissemination and transmission of virus and tumor progression. We have shown that the viral transactivator Rta is necessary and sufficient to reactivate KSHV. Rta forms ternary complexes with DNA and the effector of the Notch signaling pathway, RBP-Jk. We hypothesize that Rta reprograms the Notch signaling pathway through enhancing RBP-Jk binding to essential and highly specific DNA sequences. Our goals are to define how RBP-Jk DNA binding specifies transcriptional targets of RBP-Jk-dependent transactivators, dysregulates the Notch signaling pathway, and reprograms cellular transcriptomes.

Our preliminary data suggest that Rta-responsive promoters can be classified into 4 groups based upon architectures of Rta and RBP-Jk binding sites. To identify authentic targets of Rta/RBP-Jk complexes in infected cells, we performed a ChIP/Southern experiment with viral DNA. We analyzed Rta/RBP-Jk transactivation and DNA binding of a set of genomic fragments identified by the screen, and defined the role of Rta/RBP-Jk elements in specifying transcriptional start sites in the virus. Using a set of mutant and hybrid promoters, we demonstrated that sequence specificity of RBP-Jk sites is not the primary determinant for Rta transactivation. We are currently defining the DNA requirements that determine the gene specificity of RBP-Jk-dependent transactivators, and the molecular mechanism by which Rta stimulates RBP-Jk DNA binding, a novel level of regulation of the Notch pathway.

The Cellular Peptidyl-Prolyl Cis/Trans Isomerase Pin1 Regulates Reactivation of KSHV from Latency

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Poster N^{O}37

Kaposi's sarcoma-associated herpesvirus (KSHV) causes Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). KSHV-infected cells are predominantly latent, with a subset undergoing lytic reactivation. Rta protein is the lytic switch that reactivates virus, forming transactivation-competent complexes with Notch effector RBP-JK and promoter DNA. Rta is essential for tumorigenesis and viral replication, but is functionally inefficient, which we hypothesize is key to balancing viral oncogene expression with host cell lysis.

We previously demonstrated that Rta tetramers mediate reactivation and are determined by prolines in N-terminal leucine repeat (LR) and C-terminal regions. Strikingly, gammaherpesvirinae comparison reveals prolines constitute 18% of conserved Rta residues, and Rta is highly phosphorylated in vivo.
We hypothesize that proline-directed phosphorylation regulates Rta activity by controlling binding to peptidyl-prolyl cis/trans isomerases (PPIases). Cellular PPIase Pin1 binds specifically to phosphoserine- or phosphothreonine-proline motifs in target proteins. Pin1 dysregulation is implicated in myriad human cancers and can be subverted by viruses.
Our data show that KSHV Rta protein contains S/T-P motifs, binds directly to Pin1, is relocalized in Pin1 co-expressing nuclei and has enhanced transactivation with Pin1 at two viral promoters in uninfected cells. Pin1's effect, however, suggests a rheostat-like influence on Rta function. In infected cells, we found that endogenous Pin1 is active during reactivation and enhances Rta-dependent delayed-early gene expression and viral DNA replication. Surprisingly, Pin1 strongly inhibits late gene synthesis and infectious virion production. We thus propose that Pin1 is a unique, dose-dependent molecular timer that promotes a protective bias toward oncogenic, but non-host lytic, viral reactivation.

Elucidating the role of Notch signaling proteins in transcriptional specification during infection by a DNA tumor virus.

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Poster N^{O}38

Kaposi's sarcoma-associated herpes virus (KSHV) is the causative agent of Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castelman's disease, illnesses found predominantly in the immunocompromised. This is due to the ability of KSHV to establish a latent infection, during which no new virus is produced. Expression of the viral protein, replication and transcriptional activator (RTA), has been shown to be necessary and sufficient for entry into the lytic phase, during which new virus is produced. Our lab has shown that this protein induces the expression of viral genes by forming a complex with recombination signal binding protein-1 for Jk (Jk) and depositing Jk at viral promoters. Normally, Jk is found bound to the promoter in complex with transcriptional repressors. When the intracellular domain of Notch (NICD) is released from the cytoplasm and enters the nucleus, it displaces the transcriptional repressors bound to Jk and recruits transcriptional co-activators, inducing transcription. Over-expression of NICD1 has been shown to induce expression of a subset of viral genes, but is incapable of inducing reactivation. Based on this information, we hypothesize that this discrepancy can be explained by differential regulation of Jκ DNA binding and transactivation potency by RTA and Notch in KSHV-infected cells. Preliminary studies using our novel quantitative reactivation system confirm that wild type RTA, but not NICD1 is capable of reactivating KSHV from latency. Using this system, we also show that NICD3 and 4 are capable of inducing the production of some virus particles, but not NICD2.

Histone deacetylase classes I and II regulate Kaposi's sarcoma-associated herpesvirus reactivation.

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Poster N^{O}39

In KSHV latency in primary effusion lymphoma (PEL) cells, the promoter of the viral lytic switch gene, Rta, is organized into bivalent chromatin, similar to cellular developmental switch genes. Histone deacetylase inhibitors (HDACi) reactivate latent KSHV, and dramatically remodel viral genome topology and chromatin architecture. However, reactivation is not uniform across a population of infected cells. We sought to identify an HDACi cocktail that would uniformly reactivate KSHV and reveal the regulatory HDACs. Using HDACi with varying specificities, we found that Class I HDACi were sufficient to reactivate the virus, but differed in potency. Valproic acid (VPA) was the most effective HDACi, inducing lytic cycle gene expression in 75% of cells, while trichostatin A (TSA) induced less widespread lytic gene expression and inhibited VPA-stimulated reactivation. VPA was only slightly superior to TSA in inducing histone acetylation of Rta's promoter, but only VPA induced significant production of infectious virus, suggesting that HDAC regulation post-Rta expression has a dramatic effect on reactivation progression. Ectopic HDACs 1, 3, and 6 inhibited TPA and VPA-stimulated KSHV reactivation. Surprisingly, ectopic HDACs 1 and 6 stimulated reactivation independently, suggesting that HDAC-complex stoichiometry is critical for the switch. Tubacin, a specific inhibitor of the ubiquitin-binding, pro autophagic HDAC6, also inhibited VPA-stimulated reactivation. Immunofluorescence indicated that HDAC6 is expressed diffusely throughout latently-infected cells, but is found in the cytoplasm and nucleus during reactivation. Overall, our data suggest that inhibition of HDAC classes I and IIa, and maintenance of HDAC 6 (IIb) activity, are required for optimal KSHV reactivation.

Two-component histidine phosphotransfer protein Ypd1 is not essential for viability in Candida albicans

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Poster N^{O}40

Prokaryotes and lower eukaryotes, such as yeasts, utilize two-component signal transduction pathways to adapt cells to environmental stress and regulate expression of genes associated with virulence. One of the central proteins in this type of signaling mechanism is the phosphohistidine intermediate protein Ypd1. Ypd1 is reported to be essential for viability in model yeast Saccharomyces cerevisiae and basidiomycetous pathogenic fungus Cryptococcus neoformans. We report here that that is not the case in Candida albicans. Disruption of YPD1 causes cells to flocculate and filament constitutively under conditions that favor growth in yeast form. To determine the function of Ypd1 in the Hog1 MAPK pathway, we measured phosphorylation of Hog1 MAPK in ypd1Δ/Δ and wild type strains of C. albicans. Constitutive phosphorylation of Hog1 was observed in the ypd1Δ/Δ compared to wild type. Furthermore, fluorescence microscopy reveals that GFP-tagged Ypd1 is localized to both nucleus and cytoplasm. The subcellular segregation of GFP-tagged Ypd1 hints at important role(s) of Ypd1 in regulation of Ssk1 (cytosolic) and Skn7 (nuclear) response regulator proteins via phosphorylation in C. albicans. Overall, our findings have profound implications for mechanistic understanding of two-component signaling pathways in C. albicans.

The role of HIV capsid proteins in TLR2-mediated enhancement of HIV infection of resting CD4+ T cells

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Poster N^{O}41

The HIV capsid (CA) protein is involved in multiple stages of HIV infection, and mutations in the CA have shown to cause defective infectivity. Our laboratory previously found that TLR2 activation mediates HIV infection by enhancing viral nuclear import in resting CD4+ T cells. In this study, we explored the role of the capsid in mediating TLR2-induced nuclear import in resting CD4+ T cells. Primary resting CD4+ T cells were infected with VSV-G envelope viruses that were pseudotyped with HIV-1 provirus encoding a luciferase reporter gene and the WT or point-mutated capsid. Infected CD4+ T cells were used in a single-cycle infection assay to identify which capsid mutants have a defective response to TCR and TLR2 activation. HIV-1 ΔcPPT mutant, CA E45A or E71A mutants retained their infectivity, whereas CA P38A, K70A, and L136D mutants were defective in TLR2- and TCR-activated CD4+ T cells. Interestingly, HIV infectivity of CA T54A/N57A, R143A and Q219A mutants were impaired in primary CD4+ T cells in response to TLR2, but not TCR activation. Capsid mutations in this study did not abolish the synthesis of late RT products in TLR2-activated CD4+ T cells. However, 2LTR circles, markers for HIV nuclear import, were diminished in TLR2-activated CD4+ T cells with infection by P38A, T54A/N75A, Q63A/Q67A, K70A, E128A/R143A, L136D, and R143A CA mutants, signifying the crucial role of these residues of CA proteins in HIV nuclear import. Conversely, Q219A had greater 2LTR circles than WT in TLR2-activated cells, suggesting a block may be occurring at viral integration.

17β-estradiol inhibits HIV infection of primary macrophages through induction of interferon α

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Poster N^{O}42

Background: In the rhesus macaque model, estrogen protects against SIV while progesterone increases susceptibility to SIV. However, the role of sex hormones in HIV transmission in humans remains unclear. We examined the effect of sex hormones on HIV infection of primary HIV target cells.
Methods: PBMCs from healthy human donors were isolated by Histopaque gradient centrifugation. Monocyte-derived macrophages (MDMs) were isolated from PBMCs by a standard attachment method. CD4+ T cells were isolated by negative selection using magnetic beads. PBMCs or CD4+ T cells were activated by incubation with PHA and IL-2 or anti-CD3 Ab/anti-CD28 Ab. Cells were treated with 17β-estradiol (E2) or progesterone (P4) for 24 h before exposure to HIV pseudotyped luciferase reporter virus or primary isolates for 2 h at 37℃. HIV infection was determined by measuring luciferase activity or levels of HIV p24 in culture media by ELISA.
Results: E2 or P4 did not have a significant effect on HIV infection of activated PBMCs or CD4+ T cells. Interestingly, pretreatment of MDMs with E2 for 24 h at a concentration 10 nM significantly blocked HIV infection. Investigation into the mechanism of E2-mediated HIV inhibition revealed E2 did not affect surface expression of CD4 and HIV co-receptors nor HIV attachment. E2 blocked infection of MDMs by a co-receptor independent HIV-1VSV-G pseudotyped virus, indicating that E2 inhibited HIV at the post-entry level. Quantitative PCR analysis of HIV reverse transcription (RT) products showed E2 blocked the synthesis of late RT products. Investigation of the role of host restriction factors showed E2 up-regulated gene expression of IFNs and APOBEC3A in MDMs. Furthermore, the anti-HIV activity of E2 was abolished in the presence of IFNα neutralizing antibody. E2-mediated HIV inhibition was also not found in bone-marrow derived macrophages from IFNα receptor (-/-) mice.
Conclusion: We demonstrated that induction of IFNs by E2 blocked HIV infection at the step of late reverse transcription in MDMs. Our study offers a better comprehension of the molecular mechanism by which estrogen modulates HIV infection and provides insight into a novel strategy for HIV prevention.

SDF-1/CXCL12 induces migration of lymphocytes by a mechanism pannexin1 hemichannels dependent.

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Poster N^{O}43

In the last few years the role that pannexin hemichannels play in immune cells has received extensive attention. Our previous work showed that HIV infection induced a biphasic opening of these channels, an early opening between 5-30 min and a late opening between 48-120 h after HIV exposure. Our data indicates that opening of panx-1 hemichannels is essential for viral entry and replication, however there function in physiological conditions is unknown. Thus, we proposed that activation of specific chemokine receptors results in the physiological opening of pannexin-1 hemichannels (Panx-1) in T lymphocytes. We determined that treatment of T lymphocytes with SDF-1/CXCL12, a key chemokine in lymphocyte migration and HIV infection, induces a transient opening of Panx-1 when compared to HIV, but not connnexin43 hemichannels. Blocking Panx-1 blocked the lymphocyte migration induced by SDF-1/CXCL12, suggesting that opening of Panx-1 is essential for lymphocyte migration by a mechanism that involves local release of ATP. Alterations in migration occur in HIV infected cells but also in other PNS and CNS diseases such as multiple sclerosis (MS). Using a KO mouse model and EAE (an animal model of MS) we demonstrated that Panx-1 hemichannels are essential for migration of immune cells into the CNS and PNS. In conclusion our findings demonstrate that Panx-1 hemichannels play an essential role in HIV infection and in leukocyte migration into the CNS opening potential new therapeutic targets to block the pathogenesis of NeuroAIDS and MS.

Identification of potential clade C neutralization determinants using the unusually sensitive HIV-1 isolate MW965.

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Poster N^{O}44

The V3 domain of gp120 is the most immunogenic region of the functional Envelope (Env) spikes found on the surface of a HIV virion. As such, the majority of infected individuals develop high affinity antibodies to this region. However, in most cases these antibodies are unable to neutralize the majority of circulating isolates as the V3 loop is usually effectively masked. Therefore, elucidating the mechanisms behind gp120's resistance to broadly neutralizing antibodies (bNAbs) will provide information that will aid in the design of an effective vaccine immunogen. ConC, the virus encoded by the clade C consensus sequence, is extremely resistant to neutralization by V3 antibodies. On the other hand, the clade C tier 1a virus MW965, is the most neutralization sensitive viral isolate described to date and can be neutralized by a wide range of V3 antibodies. Compared to ConC, MW965 has a longer and more highly glycosylated V1/V2 region, which usually correlates with greater masking, and it is thus unclear why this isolate is so sensitive. In this study, we seek to identify key determinants for the unusual neutralization sensitivity of MW965. Here, through the study of chimeric Envs, we report that the V3 and V1/V2 domains of MW965 by themselves do not affect the neutralization phenotype of ConC, suggesting that these regions of MW965 are not by themselves responsible for the sensitivity of this Env to neutralizing antibodies. Additionally, we outline the next set of experiments that will be undertaken to determine which MW965 Env domain(s) are responsible for its unusual neutralization phenotype. Information gained from these studies will be extrapolated as a guide to help map the determinants of neutralization resistance in individual clade C isolates.

Second Messenger regulation of Bacterial Response Regulators

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Poster N^{O}45

Bacteria can exist in a motile state and also form non-motile aggregates also called biofilms. Vibrio cholera is one such bacterium that can transition between the motile non-infectious lifestyle to a biofilm based infectious life style in human gastrointestinal tract. This transition between motile to biofilm based lifestyle is controlled by the concentration of c-di-GMP in the cells. A higher level of cyclic-di-GMP generally induces biofilm formation and represses flagellum thus repressing motility. A lower level of c-di-GMP on the other hand induces motility and represses biofilm formation. This transition is achieved by directly regulating the transcription factors and thus transcription initiation. We have shown that c-di-GMP regulates the activity of two enhancer binding proteins (EBP) FlrA and VpsR in V. cholerae. FlrA is the master regulator of the flagellar bio-synthesis and c-diGMP inhibits FlrA. VpsR on the other hand is the master regulator required for biofilm formation and is activated by c-di-GMP.
Our goal here is to understand how c-di-GMP activates VpsR and represses FlrA both of which belong to the EBP family. To accomplish this goal we will use biochemical, genetic and X-ray crystallographic approaches. Information gained from this study will help in development of new therapeutics that target the c-di-GMP signaling pathway.

Stimulating the Phosphorelay Through Redox: A Complex of Proteins That Control Development in Bacillus subtilis

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Poster N^{O}46

Bacillus subtilis is a gram positive model organism that is capable of swimming, forming spores, developing biofilms or taking up extracellular DNA from the environment. These varied developmental states are controlled via the response regulator Spo0A. The activity of Spo0A is dependent on its phosphorylation state which is governed by a multi-component phosphorelay. The relay is tightly regulated via a host of kinases, inhibitors and phosphatases. Recently published work has shown that a protein complex of YlbF, YmcA and YaaT activate the rate of phosphotransfer between components of the relay. This work shows that the complex may be co-purified using a single tag to relatively high purity. This complex is sufficient to stimulate the rate of the phosphorelay in vitro. Based on UV-Vis spectroscopy the complex appears to be a flavoprotein with a bound Fe-S cluster. These cofactors are important in other enzymes for electron transfer. Our group has preliminary evidence that oxidation of the Fe-S cluster, inhibits activation by the complex. This evidence points to a potentially novel mechanism using oxygen concentrations to regulate bacterial stationary phase development.

Superoxide-mediated protection of Escherichia coli from antimicrobials

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Poster N^{O}47

Antimicrobial lethality is promoted by reactive oxygen species (ROS) such as superoxide, peroxide, and hydroxyl radical. Pretreatment with subinhibitory concentrations of plumbagin or paraquat, metabolic generators of superoxide, paradoxically reduced killing for oxolinic acid, kanamycin, and ampicillin. These pretreatments also reduced an oxolinic acid-mediated ROS surge. Defects in SoxS-MarA or AcrB eliminated plumbagin- and paraquat-mediated MIC increases but maintained protection from killing. Thus, superoxide has both protective and detrimental roles in response to antimicrobial stress.

Oncogenic signaling activates endogenous telomerase expression

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Poster N^{O}48

Cellular senescence is a stable proliferative arrest that functions as a barrier to cancer progression. While senescence can be triggered in response to variety of stresses and signal imbalances, previous results from our lab demonstrated that the primary reason why cells in several human cancer precursor lesions had stopped proliferating is because telomeres within these cells had become dysfunctional. Surprisingly, while telomere dysfunction induced senescence (TDIS) was previously thought to be exclusively a consequence of gradual telomere erosion that accompanies every cell division, our data demonstrated that oncogene (H-RasV12, BRaf-V600E and CDC-6) expressing cells also arrested stably as a result of telomere dysfunction. Catalytically active telomerase rescued oncogene induced senescence by suppressing telomere dysfunction and thereby destabilizing the growth arrest. Therefore,
telomerase, which is activated in over 90% of human cancers, suppresses oncogene induced telomere dysfunction. In most cases, reactivation of telomerase is a post-crisis event that leads to senescence bypass and consequently immortalization. However, the effector processes underlying the spontaneous activation of hTERT is not fully understood.

We will demonstrate that oncogene over-expression initially leads to cellular senescence that is stabilized by dysfunctional telomeres. However, after a prolonged period in a seemingly stable proliferative arrest, cells eventually emerge from senescence cultures as a consequence of spontaneous activation of endogenous telomerase (saTERT). Telomerase activity of saTERT was elevated to levels similar to those from retrovirally expressed hTERT and was sufficient to suppress telomere erosion and dysfunction in somatic human cells. In addition, we will present data characterizing the tumorigenic potential of oncogene expressing somatic cells that had bypassed senescence due to saTERT expression. Our data reveal novel insights into the mechanisms of spontaneous telomerase reactivation that is observed in over 90% of malignant human cancers.

Telomere Dysfunction As A Response To Extracellular Signals

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Poster N^{O}49

Cellular senescence is an irreversible growth arrest caused by potentially transforming events. A prominent feature of senescent cells is SASP or Senescence Associated Secretory Phenotype involving secretion of proinflammatory cytokines, extracellular matrix proteins, and degradative enzymes. This secretion develops only in context of persistent DNA damage foci and is proposed to act in an autocrine/paracrine manner to reinforce senescence. Many of these secreted factors cause premature senescence when added to the culture medium through activation of p53 mediated DNA damage response pathway. More importantly, senescent cells with dysfunctional telomeres have been found to accumulate in clusters in human precursor cancer lesions. Since secreted factors are important mediators of senescence, we wanted to test if paracrine signaling mediated by secreted factors from senescent cells is involved in causing telomere dysfunction induced senescence or TDIS.

Our data shows that young fibroblasts treated with conditioned medium from replicative senescent cells (OCM) and cytotoxic drug treated cells showed more than two fold increase in telomere dysfunction induced focus (TIF) formation. This telomere dysfunction in response to secreted factors present in the conditioned medium (CM) occurred rapidly within six hours of treatment and was stably maintained for atleast 24 hours. In telomerase expressing cells, treatment with OCM caused very low overall levels of telomere dysfunction indicating that presence of telomerase suppresses TIF formation under these circumstances. On testing two of the commonly secreted factors present in CM – IL-6 and TGF-β1 for their ability to cause telomere dysfunction, we observed that both IL-6 and TGF-β1 caused telomere dysfunction when added exogenously to the culture medium. Further experiments showed that presence of telomerase suppresses the telomeric response to IL-6 and TGF-β1. Our data demonstrates that senescent cells secrete factors that can amplify the senescence response to neighboring cells by inducing telomere dysfunction. Since TGF-β1 is also a growth factor that plays a major role during the wound healing process, we are currently evaluating the physiological significance of TGF-β1 mediated TDIS during the wound healing process.

XPB binding of p210 BCR/ABL supports disease progression in a murine model for chronic myelogenous leukemia

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Poster N^{O}50

Chronic myelogenous leukemia (CML) is invariably associated with a balanced reciprocal translocation between chromosomes 22 and 9. The major product of the rearrangement is a 210 kD in-frame fusion protein (p210 BCR/ABL). This fusion event leads to the constitutive activation of the ABL-encoded kinase activity, which is the principal driving force behind CML. However, several studies have shown that BCR encoded sequences are also necessary for BCR/ABL mediated leukemogenesis. Previous studies have demonstrated that p210 BCR/ABL interacts directly with the xeroderma pigmentosum group B (XPB) protein and that the docking site resides within the BCR component. In the present study, we have constructed a p210 BCR/ABL mutant that can no longer bind to XPB. When examined in bone marrow transplantation (BMT) model for CML, mice that express the mutant exhibit myeloproliferation with accumulated GMP population in the bone marrow, and dramatically extended lifespans relative to those that express unmodified p210 BCR/ABL. Compared to cells that express p210 BCR/ABL, an altered level of transcription is presented in cells that express the mutant. And a significantly inhibited expression level of c-MYC is observed in mutant expressing cells, which could contribute to the suppressed disease onset and progression. These results suggest that the interaction between p210 BCR/ABL and XPB may be important for p210 BCR/ABL induced CML.

New insights into the role of miR-15a/16 in early B cell development in a mouse model of chronic lymphocytic leukemia

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Poster N^{O}51

New Zealand Black (NZB) mouse is a de-novo model of chronic lymphocytic leukemia (CLL) that has been studied as a model of B-cell lymphoproliferative disorder. This mouse exhibits a point mutation six bases downstream from pre-miR–16 region on chromosome 14, similar to the one seen in human CLL. In both NZB and CLL, the disease is characterized by the presence of a malignant clone of B-1 cells expressing CD5 and B220 and reduced expression of miR-15a/16. To date neither cancer initiating population nor chronic lymphocytic leukemia stem cell has been detected for this type of leukemia. To study early stages of B cell development in the context of CLL and shed the light on the potential malignant origin of this disease we generated induced pluripotent stem cells from NZB spleen stromal fibroblasts. The pluripotency of NZB iPS cells was successfully confirmed by a number of tests including teratoma formation assay in NOD-SCID recipient mice. Our in vivo and in vitro studies on NZB iPS differentiation towards B-cell lineage cells revealed a substantial block in the maturation capacity of NZB iPS cells compared to wild type counterparts. Preliminary data suggests that exogenously delivered miR-15a/16-1 affects B-cell differentiation resulting in expression of higher levels of B220 (CD45R) and PU.1, suggesting a loss of B-1 lineage cells. In addition, miR15a has been shown to be directly or indirectly involved in the regulation of IL7Ra and Pax5 genes expression during B cell maturation. Our results support the hypothesis that NZB mouse model exhibits B1 lymphocyte restricted profile in the course of B cells development. This work will help further uncover new mechanisms of CLL development and shed light on the potential significance of miR-15a/16-1 as a B1 vs B2 lineage fate decision making factor.

Exome Sequencing in Familial IgA Nephropathy

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Poster N^{O}52

The genetic basis of familial IgA nephropathy (IgAN), a common, immune-mediated cause of kidney failure, is unknown. Familial IgAN segregates as an autosomal dominant trait with incomplete penetrance. We performed exome sequencing in 25 affected individuals from 10 well-characterized IgAN pedigrees with at least 2 or 3 affected in each family. In addition, we exome sequenced 20 index cases from 20 other well characterized IgAN families. A total of 44Mb or 1.8% of the genome was captured in each individual and subjected to Next-Gen sequencing. In total, 273,153 variants were detected and filtered through a bioinformatics pipeline. We selected variants that were not detected in control exomes and in public databases and had high-quality scores. We next prioritized single nucleotide variants (SNVs) and insertion/deletions that imparted a deleterious effect (nonsense, splice site SNVs, missense SNVs predicted to be damaging by Condel, frameshift coding indels). Finally, we prioritized genes with more than one deleterious variant that were either shared among patients in each family or present in any one of the index cases sequenced. Thus, out of 273,153 variants, a total of 53 genes with 96 variants were prioritized. Since we had linkage data available for a number of these families, all variants with a negative LOD score were eliminated. In addition variants that did not validate through Sanger sequencing and did not co-segregate with other IgAN family members were further eliminated. Out of the remaining 50 variants, 6 variants both validated and segregated with disease. Validation experiments for the remaining 44 variants are still in progress. Variants that both validate and segregate with IgAN will be further tested for frequency in ethnically matched controls. By exome sequencing 45 familial IgAN patients we have uncovered a list of candidate genes that may contribute to the pathogenesis of this complex disease that need to be further tested.

Human BDCA2+BDCA3int dendritic cells are a novel functional subtype of classical plasmacytoid dendritic cells.

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Poster N^{O}53

Dendritic cells (DCs) are a heterogeneous population critical in regulating immune responses to pathogens and self-antigens. Human peripheral blood DCs have been broadly characterized as plasmacytoid DC (BDCA2+), myeloid DC1 (BDCA1+) and myeloid DC2 (BDCA3+) subsets. Our study aimed to understand whether BDCA3+ DCs from human peripheral blood, the putative functional equivalent to murine CD8α+ DCs, constitute a homogeneous population of myeloid DC2 or if there are BDCA2+BDCA3+ (BDCA2+/3+)DCs that are functionally related to pDC. We constructed multi-color panels to phenotype lineage negative PBMC for expression of BDCA2 and BDCA3 by flow cytometric analysis. Four DC subsets characterized by their BDCA2 and BDCA3 expression were analyzed: BDCA2+/3neg (~0.21%), BDCA2+/3int (~0.22%), BDCA2neg/3int (~0.14%), and BDCA2neg/3hi (~0.03%). The functional significance of BDCA2+/3int DCs was assessed by stimulating PBMC with TLR9 agonists and staining for intracellular cytokines. At 6hrs, the stimulated BDCA2+/3int DC subset yielded more IFN-α+ and TNF-α+ cells than the other 2 subsets. All four subsets produced IFN-λ1/3 upon 8hr HSV-1 stimulation, but more of the BDCA2+/3neg and BDCA2+/3int DCs expressed IFN-λ1/3. IRF7, the master regulatory transcription factor of IFN production in pDCs, was constitutively expressed by the BDCA2+/3neg and BDCA2+/3int ¬subsets but not by the BDCA2neg subset. Both BDCA2+/3neg and BDCA2+/3int preferentially nibbled HSV-infected Raji cells (with nibbling by BDCA2+/3int > BDCA2+/3neg), while the BDCA2neg/3int/hi efficiently nibbled both uninfected and infected cells to a similar extent. These results suggest that BDCA2+/3int DCs exhibit major functions associated with classical pDCs and are phenotypically and functionally distinct from BDCA2neg/3int and BDCA2neg/3hi DCs.

TLR7 cross-talk affects Type I and Type III IFN production in primary human pDCs

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Poster N^{O}54

Human plasmacytoid dendritic cells (pDC) recognize single stranded RNA (Influenza/Flu) and double stranded DNA (HSV) viruses through endosomal TLR7 and -9, respectively, resulting in production of type I, III interferons (IFN) and pro-inflammatory cytokines. We observed that TLR7 is constitutively present in the endoplasmic reticulum and LAMP1-positive (late) endosomes in pDC, and upon stimulation with Flu, TLR7 co-localization with LAMP1-positive endosomes increased. Synthetic small molecule TLR7 ligands from the imidazoquinoline family, such as 3M003, are being used for anti-viral therapy and as immune adjuvants. We investigated the mechanisms by which 3M003 affects virus-induced IFN production in pDCs. 3M003 induced IFN-α much more rapidly than Flu or HSV, but at much lower levels than the viruses. Interestingly, 3M003 inhibited TLR7 or -9 ligand induced IFN production when co-administered to pDC in vitro along with Flu, HSV, HIV or CpGA. Adding 3M003 even 1-4hrs after flu stimulation inhibited IFN production by pDC. However, 3M003 did not affect the ability of pDCs to uptake Lucifer-yellow or FITC-dextran. In addition, we found that 3M003 blocked HSV and Flu induced IRF7 nuclear translocation and IFN-α production but enhanced IRF7 phosphorylation. Although the TLR7 inhibitor IRS661 inhibited Flu-induced IFN production, neither IRS661 or cysteine endolysosomal protease inhibitor, Z-FM-FMK inhibited 3M003 induced IFN production. Taken together, these results indicate that in pDCs, signaling pathway leading to IFN-α production in response to virus and 3M003 are different, and can be affected by crosstalk resulting from co-stimulation.

Exploring the mechanism of Env-mediated fusion between primary dendritic cells and T lymphocytes

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Poster N^{O}55

Myeloid and plasmacytoid dendritic cells comprise 0.2-0.6% of circulating peripheral blood mononuclear cells. mDC express Toll-like receptors (TLR) 2-4, and secrete IL-12, while pDC express endosomal TLR-7/9 and produce vast amounts of IFN-α upon activation. Importantly, there is a functional and numerical loss of pDC in HIV-1 infection. We have proposed one of the mechanisms of in vivo HIV-1-induced pDC depletion to be Env-mediated pDC:T cell syncytia formation. We previously have shown CD4- and CCR5/CXCR4 chemokine-receptor dependent pDC fusion with primary, acutely-infected and chronically-infected CD4 T cells. In this study, we investigated whether this process is unique to pDC and further explored interesting mechanistic aspects in vitro. We observed that mDC, similar to pDC, fuse with chronically-infected T cells and that viral replication is not necessary for pDC-T cell fusion. In addition, we observed that HIV-1 also induced fusion of pDC with an infected pDC-like cell line, GEN2.2. Interestingly, we found an enhancement of HIV-1-infected T cell fusion with TLR7/9 stimulated pDC, while pDC nibbling of infected material was decreased. However, TLR9 blockage, but not TLR7, decreased this enhancement. Similarly, fusion with infected targets increased with IFN-α pretreatment of pDC whereas pDC nibbling was decreased. Finally, pDC:T cell fusion led to apoptosis of the fused cells when compared to non-fused cells in the population. In summary, highly apoptotic Env-mediated fusion occurs between infected T-cell targets and mDC or pDC, as well as pDC can also fuse with HIV-1-infected pDC. TLR7/9 activation is involved in promoting fusion, possibly via IFN-□ production.

LPS enhances HIV-1-induced maturation and programmed death ligand-1 expression on human plasmacytoid dendritic cells

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Poster N^{O}56

Aberrant immune activation and immune exhaustion are features characteristic of chronic HIV infection. Elevated plasma LPS levels resulting from disrupted gut mucosa during HIV infection have been implicated as a major factor contributing to immune activation that is a hallmark of HIV infection. Although pDC constitute only about 0.2-0.5 % of total PBMC, this dynamic immune cell has crucial roles in moderating innate as well as adaptive immune response. Since LPS is a major contributor to HIV-induced immune activation, we undertook this study to investigate the mechanisms by which it can modulate pDC function, their PD-L1 expression and maturation. Using flow cytometry we confirmed our previous results that pDC express low levels of TLR4 and respond to LPS. We also investigated the influence of LPS on virally stimulated pDC maturation and PD-L1 expression. Using kinetic analyses, we observed that LPS enhanced HIV-1-induced expression of PD-L1, CD40, CD83, and the chemokine receptor, CCR7 expression on pDC and that PD-L1 expression was up-regulated on matured pDC. IFN-α expression preceded PD-L1 expression on pDC following HIV-1 stimulation. LPS, however, depressed HIV-1 induced CXCR4 and CCR5 expression on pDC. Together, these results suggest that pDC adopt a tolerogenic phenotype in response to HIV stimulation and LPS contributes to this.

Role of Toll-like Receptor 7 and cytokines in TRAIL expression by human plasmacytoid dendritic cells during HIV infection

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Poster N^{O}57

As potent producers of IFN-α, pDC are essential in the innate immune defense against viruses. However, during chronic HIV infection, pDC are dysfunctional. Chronic stimulation of pDC in vivo during HIV infection leads to expression of TRAIL, a death ligand. TRAIL has been implicated in bystander apoptosis of uninfected CD4+ T cells during HIV-infection, contributing to the CD4 decline during HIV infection. In this study, it was found that pDC from HIV+ subjects with more progressive infection express higher levels of soluble and membrane TRAIL than those at earlier stages of infection and healthy controls. To investigate the mechanisms of TRAIL upregulation, pDC were stimulated with HIV-MN-AT2 and analyzed for TRAIL co-expression with activation and maturation markers CD40 and CD86. pDC were treated with TLR7 antagonist IRS661 prior to HIV-MN-AT2 stimulation in order to study the role of TLR7 in TRAIL upregulation. The role of cytokine receptor signaling in the induction of TRAIL expression was also studied by treating pDC with type I and III IFN, IL-6, or TNF-α with or without HIV-MN-AT2. It was found that TRAIL expression by pDC during HIV infection is associated with an activated and mature phenotype and its expression is partially dependent on TLR7 signaling. TRAIL expression is also potentiated by co-treatment with type I and III IFN, but is not affected by IL-6 or TNF-α. In conclusion, TRAIL expression by pDC during HIV infection is regulated by TLR7 signaling and can be enhanced by type I and III IFN receptor signaling.

RTX-toxin plays the key role in Kingella kingae virulence in infant rat model

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Poster N^{O}58

Kingella kingae is a human oral bacterium that can cause infections of the skeletal system in children and infective endocarditis in children and adults. K. kingae produces a toxin of the RTX-group, RtxA. To investigate the role of RtxA in K. kingae pathogenesis in vivo, PYKK081 and its isogenic RtxA-deficient strain, KKNB100, were tested for their toxicity and virulence in 7- and 21-day old rats after intraperitoneal injections. Using the 7-day rats, we demonstrated that at the doses above 8 x 106 cells/animal the strain PYKK081 was able to cause a fatal illness development resulting in weight loss, bacteremia, and abdominal necrotic lesion formation. Histopathological examination of sick animals showed significant immunotoxic effects in rat pups thymuses, spleen, kidney, liver, and bone marrow. Strain KKNB100 was less toxic to animals and did not cause the disease at any dose tested. We have thus established an infant rat model for disease caused by K. kingae and showed that RtxA contributed significantly to the organism toxicity and is a key virulence factor for K. kingae. In contrast, in 21-day old rat pups no difference in toxicity between PYKK081 and KKNB100 was observed suggesting that the effect of K. kingae is age-specific.

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

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Poster N^{O}59

ApiA is an outer membrane autotransporter protein in Aggregatibacter actinomycetemcomitans (A.a) which is a gram negative bacterium causing a particular form of periodontal disease, termed localized aggressive periodontitis (LAP). Attachment is the first and essential step during the process of A.a infection. It is involved the function of the surface proteins. Among these proteins, ApiA is a versatile virulence factor and its various functions include binding, invasion, serum resistance, autoaggregation and inducer of cytokine release. It has been demonstrated that ApiA played a key role in the species-specific attachment of A.a to buccal epithelial cells (BECs) from human and old world primate animals. A.a mutant strain with double knockout of apiA gene and aae gene (another autotransporter protein) can not bind to BECs. Moreover, compared with conventional autotransporter protein Aae, ApiA is a trimeric autotransporter protein, which forms threefold symmetric structure. Taken together, ApiA could be a promising therapeutic target to inhibit A.a infection.
In current project, I identify c-terminal has function relationship with autoaggregation, trimeric formation and biofilm formation. Besides, combine these findings, c-terminal region is confined from 230 a.a to 295 a.a. Experiments show that E.coli with full length ApiA precipitate quickly in suspension, which is a obstacle for success of binding assay since it need homogenesis solution. Based on study of Aae structure and ApiA autoaggregation domain, we decided to create several hybrid proteins of ApiA and Aae to define binding domain and we found which is located from 35 a.a to 100 a.a.

Role of Aggregatibacter actinomycetemcomitans Virulence Factors in a Non-Human Primate Model

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Poster N^{O}60

Aggregatibacter actinomycetemcomitans (Aa) is a Gram-negative, facultative anaerobe considered to be the etiological agent of localized aggressive periodontitis (LAP), a disease characterized by massive tissue destruction and tooth loss. Aa possesses several virulence traits that are common to many mucosal pathogens; however, all effects have been shown in vitro. The aim of this study is to investigate the effect of two prominent Aa virulence traits, LuxS and leukotoxin (LtxA) in a non-human primate model. Recently, Aa strains from Rhesus (Rh) monkeys have been sequenced and show exceptional identity to human-derived Aa strains.

Our study has two goals to determine the effect of; 1) LuxS, a quorum-sensing gene that affects Aa's community organization, and 2) LtxA, a leukotoxin gene whose toxin kills lymphocytes. Aa gene knockouts were constructed in the monkey Aa strain RHAA2 employing double crossing over event. Mutations were confirmed by PCR amplification, gene sequencing, RT-PCR and biofilm formation in vitro.

Wild-type human and monkey derived Aa were compared for their ability to colonize Rh monkeys in vivo. Results showed that monkey but not human Aa can establish itself in the Rh oral cavity for extended periods. Administration of doxycycline prior to inoculation of Aa resulted in inferior colonization levels as compared to physical and local chemical debridement. Wild type Aa strain and mutants in LuxS and LtxA will be inoculated in the oral cavity of Rh monkeys to study the effect of these virulence factors in vivo in relation to host responsiveness and to Aa community organization.

Elucidation of molecular mechanisms underlying LtxA mediated cell death of WBCs

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Poster N^{O}61

Leukotoxin (LtxA), a protein toxin secreted by an oral bacterium Aggregatibacter actinomycetemcomitans is known to specifically target and kill white blood cells(WBCs).
The property of LtxA to kill WBCs is being used to study this toxin as a therapy for leukemia, lymphoma and inflammatory diseases in which LFA-1 is upregulated. LtxA binds to the receptor known as lymphocyte function antigen-1 (LFA-1), a beta 2 integrin expressed only on the surface of WBCs, followed by activation of cell death. The LtxA mediated cell death mechanism in monocytes involves both caspases and lysosomes. However, the LtxA induced death mechanism remains largely unknown for lymphocytes. To study the mechanism two molecular approaches are being used, the first involves analysis of global protein expression changes in response to LtxA, via 2D gel electrophoresis followed by mass spectrometry. The first approach identified a candidate protein Cofilin, a ubiquitous actin binding protein known to regulate actin dynamics. In both Jurkat and RL cells, cofilin underwent dephosphorylation and thus activation in response to LtxA treatment. The LtxA treatment also led to actin depolymerization as indicated by phalloidin staining of actin. The role of cofilin in actin depolymerization and LtxA killing will be further confirmed by cofilin knockdown or mutational studies. The second approach of pharmacological inhibition identified three inhibitors BAY11-7082 (NFkB inhibitor), U0126 (MEK/ERK inhibitor) and Wortmannin (PI3K/Akt inhibitor) that significantly increased LtxA mediated killing of Jurkat T cells. This suggests that survival pathways like NFkB, PI3K/Akt and MEK/ERK in Jurkat T cells play an important role in regulating LtxA mediated cell death.

Structure-function analysis of Streptococcus gordonii's α-amylase binding protein A (AbpA)

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Poster N^{O}62

Acquired enamel pellicle (AEP), composed of salivary and bacterial proteins and bacteria present on a tooth surface, plays an important role in the development of dental caries and periodontal disease. Salivary α-amylase (HSAmy) is one of the abundant salivary proteins present in the AEP, to which few of the oral bacteria can bind and are referred to as amylase binding streptococci (ABS). These ABS bacteria possess amylase binding proteins (Abps) that promote the adhesion of bacteria to enamel, which in turn can act as a receptor for secondary colonizers. Of all the Abps known, AbpA of Streptococcus gordonii has been extensively studied for its functional role in adhesion, starch metabolism and in biofilm formation but its structure is yet to be resolved. Since the structure of a protein is critical for its function, this study is focused on structural aspects of AbpA of S. gordonii to better understand its role in saliva-bacteria interaction.
Methods: The structure of AbpA predicted by homology modeling was tested by protein mutantional and biophysical studies. Five recombinant proteins of AbpA were used for HSAmy binding assay, maltoheptaose hydrolysis and SPR studies were done to determine the binding site of AbpA to HSAmy.
Results: Recombinant AbpA protein is mostly helical consistent with our homology model. The starch binding and maltoheptaose hydrolysis studies using AbpA-HSAmy complexes indicate that N- terminal domain (34-54) and C terminal (124-165) of AbpA is important for binding to HSAmy and that the AbpA binds to HSAmy distinct from the enzymatic site.
Conclusion: Our studies conclude that AbpA does not bind to the enzymatic site of HSAmy and that the interaction between AbpA and HSAmy is likely to involve the aromatic amino acid residues of AbpA. Our future studies will entail crystallization of AbpA and completing the structure determination by x- ray diffraction studies.

Similar enhancement in dentate network excitability despite marked differences in early cellular injury following fast- and standard rate concussive brain trauma

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Poster N^{O}63

The primary cause of concussive brain injury can include a wide spectrum of insults ranging from explosions in combat to falls in elderly. Accordingly, the kinematics of the trauma can vary in key features including the rate and magnitude of the insult. In the fluid percussion injury (FPI) model, the effect of peak injury pressure on neurological outcome has been well examined. However, it is unknown whether differences in the rate of rise of the injury waveform can lead to distinct cellular and physiological changes in the hippocampus. We created a novel programmable voice-coil driven FPI device capable of producing pressure waves with different rise times to address this question.

Young adult male rats (25 day) were subject to lateral FPI at a constant peak pressure (2 atm) with a rise time of either 3 ms (fast-rate) or 10 ms (standard-rate of conventional FPI). Histological and physiological studies were conducted in hippocampal slices obtained 4-6 hours and 7-10 days after FPI and in sham-injured controls. Fast-rate FPI resulted in immediate behavioral seizures and fatalities in significantly fewer rats than after standard-rate FPI. Numerous hilar neuronal profiles were labeled by the Gallyas stain indicating extensive mechanical injury after standard-rate FPI. In contrast, only a few hilar neurons were labeled after fast-rate FPI. Similarly, FluroJade staining immediately after fast-rate FPI revealed fewer degenerating neurons in the dentate hilus and CA1 compared to standard-rate FPI. One week after injury, hippocampal slices from rats subject to both fast- and standard-rate FPI showed a significant enhancement of the perforant path-evoked granule cell field responses compared to sham controls. Notably, despite the early differences cellular injury, the afferent-evoked dentate population spike amplitude measured one week after fast- and standard-rate FPI was not different.

These data demonstrate the rate of rise to peak pressure of the injury waveform plays a crucial role in determining the extent and subtype specificity of regional cell loss. Our data indicate that reduced cellular damage and improved immediate neurological outcome, may obscure the severity of neurophysiological changes after fast-rate injury. Our findings suggest that strategies used to assess and manage patients with relatively slower civilian TBI may not directly apply while evaluating patients with fast-rate, blast-related TBI.

Regulation by RhoA of rod photoreceptor axon retraction induced by retinal detachment

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Poster N^{O}64

Rod photoreceptors, in response to injury, display synaptic plasticity. Our previous studies demonstrated that the mechanism of retraction involves activation of RhoA and its downstream effecter Rho kinase (ROCK). Here, we used bright field microscopy to measure axon retraction of isolated salamander rod cells over time with or without inhibition of LIM kinase, a downstream effecter of the RhoA-ROCK pathway. Our results showed that a LIMK inhibitor (LIMKi) can block retraction: 42% decrease of initial rod axon length in controls after 7 hours, compared with only a 29% decrease using 10uM LIMKi. Nicardipine (Nc) also blocked retraction in our previous work, possibly by reducing myosin light chain (MLC) activity. There is 31% length reduction with 10uM Nc; however, combined treatment of LIMKi and 10uM Nc showed only 29% reduction, no further effect than single treatment.

To further understand the role of the RhoA-ROCK-LIMK pathway in axon retraction, we examined the activity of cofilin, which is an effecter of LIMK and regulates actin filament turnover. We created detachments of pig retina, incubated the retinal explants for 24 hours and examined both total and phosphoryated cofilin with Western Blots. Results showed that p-cofilin increased continuously over 24 hours whereas total cofilin remained relatively stable; treatment with LIMKi or the ROCK inhibitor Y27632 prevented such increases. Thus, there is a correlation between cofilin activity and retinal detachment. Since inhibition of the RhoA-LIMK pathway blocks axon retraction in rod cells, our findings suggest actin filament disassembly contributes to rod cell axon retraction.

The role of mTOR in oligodendrocyte differentiation and myelination in vivo

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Poster N^{O}65

Our previous study demonstrated that inhibition of mammalian target of rapamycin (mTOR), a downstream target of Akt, arrested oligodendrocyte differentiation at the O4+/GalC- late progenitor stage in vitro (Tyler et al., 2009). We have begun to investigate the role of mTOR in vivo using a Cre-Lox system where Cre recombinase is driven by the CNP promoter, allowing for oligodendrocyte-specific knockdown of mTOR. Currently, we are examining effects of mTOR loss on developmental myelination. At postnatal day 25, we found deficits in myelin oligodendrocyte glycoprotein (MOG) in all regions of the brain but the reduction was most pronounced in the cortex. Moreover, we observed deficits in myelin basic protein (MBP) in the cortex. We hypothesize that the developmental deficits in myelination with loss of mTOR in the oligodendrocyte lineage are due to a delay in differentiation due to lack of mTOR activity. To further investigate this possibility we have begun to examine the conditional mTOR knockouts at PND14. At this time all myelin proteins were reduced in the cortex. We are continuing our study of PND14 brains by examining expression of oligodendrocyte lineage markers.

Regenerative Responses of the Subventricular Zone Following Pediatric Traumatic Brain Injury

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Poster N^{O}66

Pediatric Traumatic Brain Injury (TBI) is a significant problem that affects many children each year. Progress is being made in developing neuroprotective strategies to combat such injuries; however, investigators are a long way from therapies to fully preserve injured neurons and glia. To restore neurological function, regenerative strategies will be required. The Subventricular Zone (SVZ) harbors dividing cells that have the potential to regenerate multiple types of brain cells after injury; therefore, we evaluated regenerative responses of the SVZ after pediatric. We used controlled cortical impact (CCI) injury to produced comparable damage to the somatosensory cortex of rats at postnatal day 6 (P6), P11, P17 and P60 and mice at P14. At both 48 and 96 hours after injury, the mitotic indices of animals injured at pediatric ages were significantly increased vs. sham operated and naïve controls and the regenerative response was more robust in the immature vs. the adult brain. A 4-marker flow cytometry panel and immunolabeling for Nestin/Ki-67/Mash1 showed increases in NSCs as well as in 2 classes of multipotential progenitors. BrdU+/Dcx+ cells were increased in the ipsilateral SVZ and parenchyma adjacent to the lesion 14 days after rat CCI. However, very few new mature neurons were seen in the lesion 28 days after injury. Altogether, our data indicate that although the immature brain is capable of mounting a robust proliferative response to CCI that includes an expansion of primitive NSCs and certain progenitors, these responses do not result in sustained neurogenesis or significant neuronal replacement.

Sorting out the cellular composition of Proneural Glioblastoma

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Poster N^{O}67

Glioblastoma (GBM) is the most common malignant brain tumor in adults. Traditional treatment modalities are largely ineffective in slowing tumor progression and most patients die within 2 years of diagnosis. GBMs are parsed into four distinct subtypes based on expression profiles referred to as Proneural, Classical, Mesenchymal and Neural. The Proneural subtype has specific genomic aberrations that include deletions and mutations of tumor suppressors (p53 and PTEN), mutations in a metabolic enzyme (IDH1) and amplifications in platelet derived growth factor receptor alpha (PDGFRα). To study Proneural GBMs we stereotactically delivered a replication incompetent retrovirus that produces PDGF-BB and Cre recombinase to the subcortical white matter of p53fl/fl PTENfl/fl transgenic mice. Tumors began as small collections of retrovirally infected cells at the injection site that expanded into large masses within 3 weeks. Genomic profiles of these tumors revealed preferentially clustering with the human Proneural subtype. Histologically these tumors recapitulated human GBMs and were largely comprised of NG2+/Olig2+/PDGFRα+/GFAP- cells. Using a 4 color flow cytometry analysis to characterize the expression of CD133, Lex, NG2 and CD140a, we found that the tumor contains a mixed population of cells with different phenotypes, including a small subpopulation of cells that resembled neural stem progenitor cells (CD133+, Lex+, NG2-, CD140a-). Similarly, the majority of cells of a line derived from PDGF expressing retroviral induced tumors were CD133-, Lex-, NG2+ and CD140a+, consistent with a OPC phenotype. This system can be used to study the lineage relationships of the cells in Proneural GBM. Supported by R01NS066955 awarded to P.C. and by F31NS076269 awarded to L.M.

Increased Vulnerability of Plasma Membrane Calcium Atpase 2 (PMCA2) Heterozygous Mice to Experimental Autoimmune Encephalomyelitis

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Poster N^{O}68

Multiple Sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disorder of the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS characterized by ascending paralysis. The histopathological hallmarks include myelin loss, neuronal death and axonal damage, especially in the spinal cord (sc). Earlier studies in our laboratory have shown a decrease in PMCA2 expression in sc neurons at onset of EAE and throughout the course of the disease. Moreover, we demonstrated that silencing of PMCA2 by siRNA leads to the death of sc neurons, in vitro. These results suggested that a decrease in PMCA2 could be a cause of neuronal death in EAE. As PMCA2 is an important calcium extrusion pump found in excitable cells including neurons, we pursued studies to determine its importance in EAE. We induced EAE in PMCA2+/+ and PMCA2-/- mice and evaluated neurological dysfunction. PMCA2-/- mice exhibited more severe neurological deficits compared to PMCA2+/+ mice, as indicated by significant differences in clinical and cumulative disease scores. In contrast, there was no difference in time of disease onset. Increased neurological disability in PMCA2-/- mice with EAE could be the result of increased vulnerability of neurons to inflammatory damage or a stronger systemic immune response. Ongoing studies are investigating these two possibilities. Preliminary results confirmed the absence of PMCA2 expression in lymph nodes, thymus and T cells. Current investigations are comparing the inflammatory reaction, apoptotic neuron number and dephosphorylated neurofilament H positive injured axons in the sc of PMCA2+/+ and PMCA2-/- mice.

Differentiating mild traumatic brain injury from post-traumatic stress disorder: Risk factors

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Poster N^{O}69

A significant proportion of Traumatic Brain Injury (TBI) cases are classified as mild according to the American Congress of Rehabilitation Medicine (ACRM): any period of loss of consciousness, retrospective or prospective memory impairment for the event, alteration in mental state, which does not exceed proscribed limits in duration and intensity. PTSD is conceptualized as an interaction of risk (e.g., genetics, early life experiences, temperament) and traumatic experiences to induce sequelae conforming to heighten arousal, re-experiencing, and avoidance. However, our laboratory is consistently finding reduced acoustic startle responses (ASRs), which persist at least 28 days after a single impact (17-20 psi) directed to the parietal lobe. Here, we examine whether a diathesis of temperament and exposure to lateral fluid percussion injury induce changes in stress and startle reactivity consistent with PTSD in rats. Inbred Wistar-Kyoto (WKY) rats express inhibited temperament (reduced open field reactivity), enhanced stress (exaggerated corticosterone (CORT) responses) and sensory reactivity (greater ASRs) compared to outbred Sprague Dawley (SD) rats. The inherently greater ASRs could allow the WKY to be more resilient to mTBI. WKY and SD rats were matched for basal ASR within strain and randomly assigned to either mTBI or SHAM operated conditions. ASRs were further assessed at post-injury day (PID) 4, 8 and 11 and 28. Stress reactivity was assessed as basal and CORT responses (15, 30 and 60 min) after a single 2.0 mA foot-shock on PID 7 and 10. On PID 28, brains samples were obtained 90 min after ASR test for the assessment of brain activation. As expected, SHAM WKY exhibited greater ASRs compared to SHAM SD rats. Confirming our previous findings, mTBI SD rats showed prolonged and pronounced attenuation of ASRs compared to SHAM SD rats. Consistent with SD rats, mTBI WKY rats exhibited persistent and pronounced reduction of ASRs at all time points. In fact, taking greater levels of baseline ASRs into consideration WKY rats had greater degree of post-injury reduction. As opposed to sensory reactivity, injury did not show any effect on CORT reactivity. Contrary to our expectations, WKY rats did not exhibit signs of resiliency in mTBI.

Differential enhancement of the conditioned response in delay compared to long delay eyeblink conditioning in anxiety vulnerable adolescents

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Poster N^{O}70

Individuals who are behaviorally inhibited, a risk factor for anxiety disorders, demonstrate facilitated eyeblink acquisition at optimal conditioned stimulus (CS) durations (500-ms). Developmental research in infants and older adults suggests optimal CS durations may vary with age. However, the few parametric studies available are limited to short (100-500-ms) presentations of the CS. Given this, the present study was motivated to 1) understand how longer CS durations affect eyeblink acquisition in a sample of healthy adolescents and 2) assess individual differences in acquisition of adolescents with behaviorally inhibited temperament. 94 (ages 13-17, M=15.3 years, 50% male) participants filled out a battery of anxiety vulnerability measures including the Adult Measure of Behavioral Inhibition (AMBI) prior to undergoing eyeblink conditioning. Using a median split, participants were separated into high or low vulnerability groups using scores on AMBI. Participants were randomly assigned to 500-ms or 1000-ms paired conditioning with 60 CS-US trials (1000-Hz tone co-terminating with a 50-ms 5 psi corneal airpuff unconditional stimulus (US). The percent conditioned response (CR) was calculated for each block of 10 trials. A 2 (condition: 1000, 500) x 2 (vulnerability: high AMBI, low AMBI) x 6 (block) mixed measures ANOVA revealed a significant three way interaction of vulnerability x condition x block, $F(5,450)=2.568$, $p=.026$ indicating faster acquisition of the high AMBI in the 500-ms condition. Overall, individuals acquire the CR better at 500-ms compared to 1000-ms, supporting research in other age cohorts. Similar to previous research, vulnerable individuals demonstrate facilitated acquisition at 500-ms. However, at a longer CS duration demanding increased hippocampal involvement, the facilitation is less apparent. This data with anxiety vulnerable adolescents parallels hippocampal lesion studies in rats demonstrating facilitated acquisition with short delay and impairment at long delay and trace contingencies.

Differential Processing of Neutral Stimuli in Prefrontal Regions in an Animal Model for Anxiety Vulnerability

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Poster N^{O}71

Studies have long shown differential processing of threat-related stimuli in anxious and anxiety-vulnerable individuals. It is becoming increasingly clear, however, that these processing abnormalities extend to neutral stimuli as well. Vulnerable populations may be unable to adequately modulate attention to even neutral environmental events, potentially exacerbating anxiety symptom pathogenesis. This project investigated this by assessing the effects of simple auditory stimulus presentations on behavior and neural activation in an animal model for anxiety vulnerability, the Wistar-Kyoto (WKY) rat. Sprague-Dawley (SD) controls and WKY rats were exposed to zero, one, or thirty presentations of an auditory conditioned stimulus (CS) and then subjected to delay eyeblink conditioning (EBC), pairing that CS with periorbital stimulation, or processed for c-Fos-related immunoreactivity, a marker of neuronal activation. Strain-dependent effects of CS exposure were found in both behavior and region-specific neural activation. Thirty CS exposures impaired acquisition of the EBC conditioned response ($F(2,32)=3.283$, $p=0.05$) and suppressed activation in the anterior cingulate cortex ($F(2,13)=4.226$, $p=0.039$) of SD controls, but not anxiety-vulnerable WKY rats. These results suggest that suppression of EBC by CS pre-exposure, a normal learning effect known as latent inhibition, may be contingent on the capacity to down-regulate neural activation following repeated stimulus presentations. Moreover, the region implicated by the immunohistochemical results points to the behavioral effect being driven in part by top-down attention modulation which may be impaired in anxiety-vulnerable WKY rats. These findings support the hypothesis that anxiety-vulnerable populations exhibit abnormal processing of neutral, in addition to threatening, environmental stimuli.

Human Avoidance in the Context of Anxiety Disorders: Behavioral and Computational Approaches

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Poster N^{O}72

Human Avoidance in the Context of Anxiety Disorders: Behavioral and Computational Approaches
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Avoidance behavior is a predominant symptom in all anxiety disorders. The propensity to acquire and express such behavior might be linked to specific factors that increase vulnerability to anxiety disorders. To date, a full understanding of how avoidance behavior is exhibited by humans with different personal characteristics is limited by the absence of appropriate tasks. Hence, in our first experiment, we tested undergraduate students on a computer-based escape-avoidance task, where subjects might learn that some signals predict an on-screen aversive event. Results revealed that while almost all participants learned to escape the aversive event, only two-thirds also exhibited avoidance responses. Crucially, two known vulnerability factors for anxiety disorders were differentially associated with enhanced avoidance behavior. Specifically, individuals with harm avoidant personality tended to show higher avoidance rates and females were found to exhibit more persistent avoidance (i.e., longer duration.) In a second experiment, we modified our task to see how adding a non-threat ("safety") signal during the acquisition phase affects behavior (signal was absent during extinction.) Females again showed more persistent avoidance responses than males. Furthermore, the addition of a signal that predicts a period of safety during the acquisition phase facilitated the extinction of the avoidance responses. Lastly, a computational modeling approach, as a tool for better understanding of the underlying neural mechanisms of this human behavior, will be presented. Taken together, this work sheds light on personality and sex differences that may mediate avoidance behavior and anxiety disorders. Furthermore, the reported findings might facilitate the development of personalized strategies to treat and/or prevent the development of anxiety disorders.
This work was supported by Award Number I01CX000771 from the Clinical Science Research and Development Service of the VA Office of Research and Development, by the NSF/NIH Collaborative Research in Computational Neuroscience (CRCNS) Program, by NIAAA (R01 AA018737), and by additional support from the SMBI. Authors also want to thank S. Shikari, J. Ostovich, B. Ekeh and Y. Ebanks-Williams for assistance with data collection and A. A. Moustafa for consulting on computational modeling.

Anxiety Vulnerable Rats Exhibit Abnormal Synaptic Plasticity in the Basolateral Amygdala to Prelimbic Cortex Projection

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Poster N^{O}73

Individuals that develop an anxiety disorder are believed to possess underling vulnerabilities that put them at risk for developing a particular disorder. Despite this, few animal models of anxiety account for vulnerability. The basolateral amygdala (BLA) and prelimbic cortex (PL) are two interconnected brain regions implicated in the neuropathology of anxiety. In non-vulnerable rat strains, NMDA-dependent long-term potentiation (LTP) is induced in PL following high frequency stimulation of the BLA. This potentiation can be inhibited by stressor exposure. It is unclear if an anxiety vulnerable rat strain would express similar plasticity or if anxiety vulnerability is associated with impaired LTP in the absence of a stressor. To address this question, we evaluated synaptic plasticity in the projection from the BLA to PL, in a rodent model of anxiety that accounts for vulnerability, the Wistar Kyoto rat (WKY). In this study, synaptic plasticity of BLA-evoked field potentials, generated within layers II and III of PL were assessed in WKY and non-vulnerable Sprague Dawley (SD) rats under urethane anesthesia. While WKY rats exhibited normal paired-pulse plasticity, they did not maintain LTP. This result is likely specific to BLA-evoked responses generated within layer II of PL and may results from post-synaptic abnormalities. Possible mechanism by which WKY rats fail to maintain LTP in the BLA-PL projection will be explored. However, these results suggest that activity-dependent synaptic plasticity within the BLA-PL projection is abnormal in WKY rats, and consequently may affect the processing of stimuli resulting in the anxiety vulnerability expressed by this strain.

Differentiating mild traumatic brain injury from post-traumatic stress disorder: Sensory and stress reactivity

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Poster N^{O}74

While the rate of concordance between mTBI and PTSD is high, it is not known whether having sustained an mTBI makes a patient more vulnerable to developing and expressing symptoms of PTSD. Hyper-arousal – a core symptom of PTSD – manifests as an exaggerated startle response to brief yet sharp white noise stimuli. We examined hyper-arousal by measuring the acoustic startle reflex (ASR) after mTBI in rodents. Additionally, we assessed pain sensitivity and locomotor activity to examine somato-motor effects of mTBI. Using fluid percussion, rats were injured at 21.5±1.3psi and in the absence of gross brain damage, demonstrated apnea (12s) and transient loss of consciousness (8.5min). No differences between groups were observed in pain sensitivity or locomotor activity. However, mTBI subjects showed a profound attenuation in ASR compared to SHAM, evident at 24hr post-injury and lasting up to 4 weeks post-injury. While response sensitivity was reduced to 80% of pre-injury levels, response magnitude was greatly attenuated to 20-25% across all three levels of acoustic stimuli. Stress reactivity after mTBI was also examined by measuring corticosterone (CORT) and neurosteroid levels in response to a stressor. SHAM and mTBI subjects did not exhibit any differences in serum CORT. However, serum pregnenelone, allopregnanolone and androsterone were increased in mTBI subjects compared to SHAM. These levels were also increased in the prefrontal cortex, hippocampus and cerebellum. We conclude from our results of sensory and stress reactivity that mTBI does not produce functional outcomes that are consistent with PTSD.

Selective Defects in Arcuate Nucleus (ARC) Leptin Signaling in P4 and P7 DIO Rats

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Poster N^{O}75

We have shown that offspring of rats selectively bred to develop diet-induced obesity (DIO) when fed a 31% fat, 25% sucrose diet have decreased leptin-induced phosphorylation of STAT3 (pSTAT3; a marker of leptin signaling) in the ARC and blunted ARC-paraventricular nucleus (PVN) axonal outgrowth compared to diet resistant (DR) rats as early as P10. To determine how early this decrease in leptin signaling occurs postnatally, we assessed ARC leptin-induced pSTAT3 expression in P4 DIO and DR neonates. DIO neonates had 33% more ($F(1,14)=8.40$, $p=0.01$) pSTAT3-positive neurons in the rostral and 22% more in the caudal ARC $F(1,15)=7.31$, $p=0.02$). But, overall, there were no differences from DR rats throughout the entire ARC. Although P4 DR and DIO rats had the same total number of POMC neurons and POMC neurons expressing pSTAT3, DIO rats had 23% fewer POMC neurons that expressed pSTAT3 selectively in the rostral ARC ($F(1,11)=13.16$, $p=0.004$) . However, by P7, DIO neonates had 19% fewer leptin-induced pSTAT3 expressing neurons through the entire ARC ($F(1,13)=6.18$, $p=0.03$), with the largest decrease (32%) in the caudal ARC. Therefore, while DIO neonates have similar overall leptin-induced ARC pSTAT3 at P4, they have a selective reduction of leptin signaling in a subpopulation of POMC neurons and an overall reduction in ARC pSTAT3 expression by P7. These data suggest that DIO rats have an early, selective postnatal defect in leptin receptor signaling that continues throughout life and is likely to predispose them to become obese on a high fat diet.

Perifornical Hypothalamic Serotonin Regulates the Counterregulatory Response to Hypoglycemia

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Poster N^{O}76

To identify potential sites of action where serotonin (5HT) modulates the counterregulatory response (CRR) to insulin-induced hypoglycemia (IIH), we first evaluated 5HT turnover (ratio of 5-hydroxyindole acetic acid, 5HIAA, to 5HT) in rats (n=6/group) treated for 6d with the selective 5HT reuptake inhibitor, sertraline (SERT, 7.5 mg/kg/day, s.c.), or vehicle (VEH, 50% ethanol) by osmotic minipump. This regimen was previously shown to enhance the epinephrine response to IIH (30-40mg/dL glucose over 2h after 4.5 U/kg insulin, s.c.). In SERT-IIH rats, 5HIAA/5HT was significantly lower than VEH-IIH rats in the perifornical hypothalamus (PFH, 0.968±0.065 vs. 1.258±0.052; p=0.024) and paraventricular thalamus (1.53±0.21 vs. 2.39±0.51; p=0.018). As an IIH surrogate, we next evaluated the hyperglycemic response to 2-deoxy-D-glucose (2-DG, 200 mg/kg, s.c.), after selective bilateral PFH 5HT axon terminal ablation with the 5HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 4 µg in 0.5 µL), vs. VEH (0.1% ascorbic acid). Relative to VEH controls, PFH 5,7-DHT lesions reduced the CRR to 2DG by 61% (2h glucose AUC: 19508.6±359.5 mg/2h, n=10 vs. 11889.7±1164.1 mg/2h, n=6; p<0.001). On the other hand, bilateral PFH 5HT-1A receptor agonism with 8-OH-DPAT (5 nmol in 0.5 µL saline) amplified the CRR to 2DG by 22% (8-OH-DPAT: 17548.8±781.4 mg/2h, n=7; VEH: 13744.1±489.1 mg/2h, n=8; p=0.001). Therefore, systemic sertraline administration reduced the 5HT turnover response to IIH in discrete diencephalic regions previously identified as potential components of the brain CRR network. Additionally, in the PFH, 5HT depletion blunts, while 5HT-1A receptor agonism amplifies the CRR to 2-DG.

Relationship between Posttraumatic Stress Disorder and Vestibular Function

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Poster N^{O}77

Background: Posttraumatic stress disorder (PTSD) is common in veterans and associated with a number of symptoms including dizziness, a symptom of PTSD that impairs function [1]. Furthermore, clinical data from our center demonstrates increased reports of symptoms of dizziness among PTSD patients. Dizziness symptoms are also highly associated with vestibular dysfunction [2-4]. Despite this, current PTSD assessments do not include an assessment of vestibular function. Therefore, our aim is to determine whether there is an increased incidence of vestibular impairment among PTSD patients.
Methods: A group of 27 veterans (Age 27 to 58) were evaluated at the War Related Illness and Injury Study Center (WRIISC), East Orange VA for vestibular function. Vestibular function was assessed by posturography and rotational chair testing. PTSD was classified based on scoring 44 or higher on the posttraumatic checklist civilian (PCL-C). Head injury status was determined from a level two polytrauma interview.
Results: Ten veterans were classified as having PTSD. Examination of posturography demonstrated that in Veterans with PTSD they had significantly poorer equilibrium scores on SOT2 (eyes closed), Controls – 91.7±3.5 vs PTSD – 85.9±6.5, $P<0.05$. In contrast there was no difference in the SOT5 condition (eyes closed, unstable platform). Consistent with no differences in SOT5, the vestibular ratio was also not different between groups (Controls – 0.54±0.30 vs PTSD - 0.52±0.22). Consistent with this, ocular counter-roll (OCR), an otolith mediated vestibular ocular reflex was also not different between groups (Controls – 0.13±0.06 vs PTSD - 0.18±0.07).
Conclusion: Our preliminary data indicate that veterans with PTSD appear to have normal posturography and normal balance. This was in contrast to our original hypothesis that vestibular dysfunction may be more prevalent in veterans with PTSD. One possible confounder is the effect of mild TBI on vestibular function. However, controlling for mTBI did not affect our results. Another possible explanation is that veterans with PTSD may have canal rather than otolith dysfunction. Thus, future work is necessary to examine both otolith and canal function in this population.
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Ventromedial hypothalamic (VMH) glucose sensing neurons are sexually dimorphic and are regulated by 17β-estradiol via pre- and postsynaptic mechanisms.

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Poster N^{O}78

Reduced plasma estrogen levels increases a women's risk of developing metabolic syndrome. 17β-estradiol (17βE) acts centrally to promote negative energy balance potentially via membrane-associated estrogen receptor (ER) signaling in regions of the brain associated with energy balance such as the ventromedial hypothalamus (VMH). VMH glucose sensing neurons (GSNs) sense glucose deficit and are thus a potential target for the central effects of 17βE. These neurons either increase (glucose-inhibited, GI) or decrease (glucose-excited, GE) their action potential frequency in low glucose. The VMH contains the arcuate (ARC) and ventromedial (VMN) nuclei. ARC ERs are widely dispersed, but VMN ERs and GSNs are concentrated ventrolaterally (VL). Female mice lacking ERα in steroidogenic factor-1 (SF1 ERα-/-) neurons, an VMN exclusive marker, display profound metabolic disturbances while male SF1 ERα-/- mice are normal. Hypoglycemia counterregulation (CRR) differs in females vs males. This suggests sex differences in 17βE regulation of energy homeostasis. We hypothesize that glucose sensitivity and/or estrogen regulation of VMN GSNs differs between the sexes. To test this, we used whole-cell electrophysiological recordings of VL-VMN GSNs in brain slices from prepubescent (3-4 week old) male and female mice. In 2.5mM glucose, 17βE (100nM) depolarized and increased input resistance (IR) in VL-VMN GI and GE neurons in both sexes to a similar degree. In VL-VMN GI neurons, the 17βE effect reversed at -88±3mV and -87±2mV in females (n=8) and males (n=6), respectively, suggesting that 17βE utilizes a similar excitatory mechanism in both sexes. This mixed reversal potential is dominated by K+ conductance (EK+=-99mV), but may contain other components. The excitatory effect of 17βE in 2.5mM glucose did not persist in tetrodotoxin (500nM, TTX) suggesting a presynaptic mechanism. In females, low glucose (2.5□0.1mM) increased IR by 52±6% (n=28) in VL-VMN GI neurons and decreased IR by 36±2% (n=9) in VL-VMN GE neurons compared to 72±15% (n=12, p=0.02) and 52±4% (n=5, p=0.002) for VL-VMN GI and GE neurons, respectively, in males. These data suggest that VL-VMN GSNs are less sensitive to glucose decreases in females vs males. In addition, 17βE significantly blunted the response of VL-VMN GI neurons to low glucose in both sexes (♀n=6, ♂n=3). In females, the effect of 17βE on glucose sensitivity of VL-VMN GI neurons persisted in TTX suggesting that this aspect of estrogenic regulation is postsynaptic. In GI neurons, phosphorylation (p) of AMP-activated kinase (AMPK) is critical for activation in low glucose. In females, both 17βE and bovine serum albumin-conjugated estrogen (100nM, membrane ER agonist) stimulated VMH pAMPK in 2.5mM glucose, but blocked pAMPK in response to low glucose. In males, 17βE had no effect on the glucose sensitivity of VL-VMN GE neurons (n=3); however this has yet to be evaluated in females. To further differentiate direct vs indirect effects of 17βE, we utilized post-recording immunohistochemistry (pr-IHC) and single-cell PCR (scPCR) to evaluate ERα expression in VL-VMN GSNs. Via pr-IHC, 11% (5:44) and 20% (2:10) of VL-VMN GI and GE neurons expressed ERα protein, respectively. In addition, via scPCR, 16% (2:12) and 33% (2:6) of VL-VMN GI and GE neurons expressed ERα mRNA, respectively. This suggests that only a subpopulation of VL-VMN GSNs expresses ERα mRNA or protein and supports the possibility that 17βE utilizes pre- and/or postsynaptic mechanisms. In conclusion, the glucose sensitivity of VL-VMN GSNs is sexually dimorphic. Furthermore, 17βE may acts via pre- and postsynaptic mechanisms to influence VL-VMN GSNs neuronal excitability and VL-VMN GI glucose sensitivity. Thus, sex differences in glucose sensing may contribute to the increased susceptibility for metabolic syndrome in women with altered estrogen levels as well as sex differences in hypoglycemia counterregulation.

N-acetylcysteine corrects glucose sensing of ventromedial hypothalamus (VMH) glucose-inhibited (GI) neurons following recurrent hypoglycemia or diabetic hyperglycemia in rats

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Poster N^{O}79

Intensive insulin therapy is necessary for patients with type 1 diabetes mellitus (T1DM) in order to avoid the complications of hyperglycemia. However, hypoglycemia occurs when insulin administration exceeds that which is needed to maintain euglycemia. Recurrent hypoglycemia (RH) impairs the autonomic and neuroendocrine counterregulatory responses (CRR) that normally restore euglycemia. This is especially problematic because T1DM per se also impairs the CRR placing these patients at a high risk for dangerous hypoglycemic episodes. Both diabetic hyperglycemia and RH blunt the activation of glucose-inhibited (GI) neurons in the ventromedial hypothalamus (VMH) by decreased glucose. The effects of diabetic hyperglycemia and RH on VMH GI neurons may be mediated by the increased oxidative stress resulting from reducing thioredoxin and glutathione (GSH) antioxidant defense systems. One possible mechanism by which oxidative stress could impair glucose sensing is through increased S-nitrosation of the nitric oxide (NO) receptor guanylyl cyclase (sGC) which reduces its affinity for NO. NO signaling via sGC is critical for glucose sensing in VMH GI neurons as well as for the CRR. Consistent with this hypothesis, we find that RH increases VMH sGC s-nitrosation. Moreover, increasing GSH levels with N-acetylcysteine (NAC) reduces VMH sGC s-nitrosation and prevents the impaired CRR after RH. Therefore, we hypothesized that NAC will also prevent the impairment of glucose sensing by VMH GI neurons following RH and/or diabetic hyperglycemia. RH was induced by 3 consecutive days of insulin injections and T1DM was induced by intraperitoneal injection of the pancreatic beta cell toxin, streptozotocin (STZ). NAC was given for 9 days prior to and during the RH protocol and for 12 days post-induction of diabetes. Glucose sensing by freshly dissociated VMH GI neurons was monitored using the FLIPR membrane potential dye assay in which increased fluorescence indicates neuronal depolarization. Glucose sensitivity was quantified as the %VMH neurons which depolarize in response to a glucose decrease from 2.5 to 0.1 mM. RH decreased the %VMH GI neurons compared to the saline control (saline/-NAC: 7.6 ± 0.5, n=7; RH/-NAC: 4.2 ± 0.9, n=7; $P<0.05$). After NAC pre-treatment there was no longer a difference between RH and saline controls (saline/+NAC: 6.5 ± 0.6, n=7; RH/+NAC: 6.5 ± 0.4, n=10; $P>0.05$). T1DM also decreased the %VMH GI neurons compared to vehicle injected controls (vehicle/-NAC: 5.2 ± 0.5, n=10; STZ/-NAC: 1.1 ± 0.3, n=10; $P<0.05$). Like RH, NAC treatment reversed the effect of T1DM on the response of VMH GI neurons to decreased glucose (STZ/+NAC: 3.3 ± 0.5, n=9; $P<0.05$ compared to STZ/-NAC; $P>0.05$ compared to vehicle/-NAC or vehicle/+NAC [5.0 ± 0.6, n=11]). However, when T1DM rats were exposed to RH, NAC treatment was no longer able to restore the response of VMH GI neurons to decreased glucose (% GI in STZ/RH/-NAC: 1.7 ± 0.4 vs. STZ/RH/+NAC: 2.8 ± 0.5; n=14, $p = 0.15$). Therefore, we conclude that by enhancing GSH antioxidant defense, NAC restores the response of VMH GI neurons to decreased glucose in rats exposed to RH or T1DM alone. However, NAC is not sufficient to restore normal VMH GI glucose sensing when T1DM and RH occur in combination. This suggests that enhancing the thioredoxin antioxidant defense in addition to GSH system might be necessary in order to fully restore glucose sensing and the CRR when RH occurs during T1DM.

Unraveling the role of calcium in AA-induced mTORC1 activation

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Poster N^{O}80

Diabetes is a worldwide problem today and 95% of the diabetic cases are caused due to obesity (nutrient overload and inactivity). Recently excess protein consumption resulting in increased circulating levels of amino acids (AAs) has been shown to induce insulin resistance and disturb glucose homeostasis along with other nutrients like glucose and fats. Studies have also shown that AA overload induces insulin resistance by constitutively activating mammalian target of rapamycin complex 1 (mTORC1/S6K1) which desensitizes insulin signaling pathway. The pathway taken by AAs in inducing S6K1 activation is being extensively explored and previous studies from our lab and other research groups suggests the involvement of calcium in AA-induced mTORC1 activation. But the underlying molecular mechanism by which AA overload causes activation of S6K1 is yet to be discovered. In the present study, we have confirmed the involvement of calcium in AA-induced S6K1 activation/phosphorylation. In order to determine the role of calcium, western blot experiments to measure phosphorylation of S6K1 at Thr389 position was performed on HEK cells deprived of AAs and serum for two hours. HEK cells were repleted with AAs for 30 minutes during the course of the experiment. In HEK cells repleted with AAs there is an increase in phosphorylation of S6K1 when compared to the unrepleted cells. This increase in phosphorylation of S6K1 on AA repletion was suppressed when cells were pretreated with an intracellular calcium chelator BAPTA-AM (50µM) suggesting a role of calcium in AA-induced S6K1 activation.

Role of GLUT5 and Fructokinase in the activation of glucosensing neurons of the ventromedial hypothalamus.

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Poster N^{O}81

The ventromedial hypothalamus (VMH) in the brain is critical for the regulation of blood glucose levels. Two main populations of glucose sensing neurons (GSNs) known as glucose-excited (GE) neurons and glucose-inhibited (GI) neurons are known to participate in the control of both food intake and the counter-regulatory response (CRR) to hypoglycemia. In a mechanism that is apparently similar to the response of GI neurons to glucose deprivation, fructose catabolism via fructokinase leads to depletion in cytosolic ATP contents, consequently increasing AMP/ATP ratio and activates fuel sensor AMPK. Thus, high fructose mimics the effect of low glucose in activating GI neurons.
Using calcium imaging as an indicator of neuron depolarization and activation, we showed that fructose can directly stimulate (called Fructose, or F-activated neurons) or inhibits (called F-inhibited neurons) VMH neurons. Fructokinase (KHK) knockout mice showed almost abolished fructose effects while GLUT5 knockout mice had a significantly decreased response to fructose. The stimulating effect of fructose is dependent on AMPK, neuronal nitric oxide synthase (nNOS) and the cystic fibrosis transmembrane conductance regulator (CFTR) channel, and largely overlaps with GI response. Studies using in vivo fructose injection towards the brain caused a transient increases in blood glucose concentration, and stimulated glucagon release in euglycemic rats.
Thus we hypothesized that fructose can act centrally in the VMH as a result of transport by GLUT5 and metabolism via fructokinase to elicit responses normally elicited by hypoglycemic at the level of blood glucose and glucagon level.

Role of metabolism in fructose-induced GLUT5 regulation

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Poster N^{O}82

Marked increases in fructose consumption have been linked to increases in prevalence of metabolic diseases including obesity and diabetes. Fructose upregulates its own absorption by increasing the expression and activity of the intestinal fructose transporter GLUT5, but the underlying mechanism for this regulation is not completely understood. Defects or limits in regulation can lead to adult-onset fructose intolerance or to fructose malabsorption in infants. I tested the hypothesis that fructose transport via GLUT5, metabolism via ketohexokinase (KHK), and GLUT5 intracellular trafficking to the apical membrane via the GTPase Rab11a are required for GLUT5 upregulation. Adult wildtype, GLUT5-/- and KHK-/- mice were divided into three groups each receiving 30% glucose, fructose or lysine by gavage twice a day for three days. Postweaning wildtype and Rab11a-/- mice were divided into two groups and gavaged with either 30% glucose or fructose. Afterwards, mucosae were collected from the small intestine then GLUT5 activity as well as mRNA and protein expression determined. Activity and expression of GLUT5 and of other enzymes involved in fructose metabolism increased in fructose-gavaged mice compared to those gavaged with glucose or lysine. This effect was specific because expression of unrelated transporters like SGLT1 did not change. Strikingly, fructose failed to induce the expression and activity of GLUT5 and of other enzymes involved in fructose metabolism, in KHK-/-, GLUT5-/- and Rab11a-/- mice. Thus, I have demonstrated for the first time the essential roles of membrane transport, metabolism, and Rab11a-mediated trafficking in fructose induced GLUT5 upregulation. (NSF IOS-1121049)

Anti-inflammatory effects of 18β - Glycyrrhetinic acid and Carbenoxolone are independent of connexin and pannexin channels.

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Poster N^{O}83

A pathologic hallmark of neurodegenerative disorders like Amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease is reactive microgliosis which is characterized, among others, by the accumulation of activated microglial cells. These microglial cells are the macrophages of the brain, resident immune cells of the central nervous system, which serve as the first line of defense in case of any type of brain injury. Activated microglia releases various factors such as tumor necrosis factor-α (TNF- α), nitric oxide (NO), interleukin-1β (IL-1β), Prostaglandin (PGE-2), glutamate and ATP, which in excess are detrimental, and thus contribute to chronic neuroinflammation and neurodegeneration. In microglia and macrophages, the potential gates for the release of transmitters, such as ATP and glutamate, to the external milieu are attributed to the coordinated action of specialized channels called connexin and pannexin hemichannels1, specifically Connexin 43 (Cx43) and Pannexin 1 (Panx1) in microglia and macrophages cells. Hence, blockade of hemichannels is potentially beneficial in treatment of neurodegenerative diseases. Several recent studies suggested that 18β-glycyrrhetinic acid (18β-GA) and its derivative, carbenoxolone (CBX), have neuroprotective actions via the blockade of pannexin and connexin hemichannels and, consequently, more studies are focusing on the therapeutic use of these hemichannel blockers. Here, we begin to examine whether these blockers have an anti-inflammatory effect, preventing microglia/macrophage activation via closing connexin43 (Cx43) and/or pannexin1 (Panx1) channels, both of which are expressed in activated microglia and/or macrophages.

Structure-function studies and signaling mechanisms of the D3 dopamine receptor

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Poster N^{O}84

Dopamine is a member of the biogenic amine family of neurotransmitters and improper regulation of dopaminergic transmission results in disorders such as Parkinson's disease, schizophrenia and addiction. Dopamine receptors are G-protein coupled receptors (GPCRs) and are classified as D1-D5. Although the D3 dopamine receptor (D3R) shares high homology with D2, it possesses distinct signaling properties when repeatedly stimulated with agonist. The D3R exhibits two unique signaling properties, tolerance and slow response termination (SRT) to dopamine and other D2-like receptor agonists. Tolerance is defined as a reduction in signaling response upon a second exposure to agonist and SRT is the slow termination of the response upon agonist removal. Structure-function studies from our lab have determined that the intracellular loop-2 is necessary but not sufficient for the tolerance property of the receptor and the regions of the D3R contributing to the phenomena of tolerance and SRT are different for different D3R agonists. This led us to hypothesize that there are other regions apart from the intracellular loops of the D3R involved in the tolerance and SRT properties of the D3R. In addition to identifying the structural domains involved in the tolerance and SRT properties of the D3R, there is a need to develop and refine the agonist-bound D3R pharmacophore model. We have chosen site-directed mutagenesis approach coupled with molecular docking and molecular dynamics simulations to identify the critical amino-acid residues involved in the agonist-induced activation of the D3R. We have so far identified C147 residue in the intracellular loop-2 to be important for the tolerance property of the receptor to the agonists dopamine and quinpirole while D187 residue in the extracellular loop-2 to be important for the tolerance property of the receptor to the agonists PD128907, quinpirole and dopamine. Molecular dynamics simulations suggested that the D3R adopts a different conformation in the tolerant state and the H354 residue in the extracellular loop-3 forms a salt bridge with the D187 residue. We have also found that the site-directed mutation of the H354 residue resulted in loss of PD128907-induced D3R tolerance supporting the modeling studies. Another goal of the current project is to elucidate the signaling mechanisms underlying the slow response termination property of the D3R. We hypothesize that the regulators of G-protein signaling (RGS) proteins with the GDI (guanine nucleotide dissociation inhibition) activity might play a role in the D3R SRT property and are currently testing the hypothesis.

Regulation of the ion channel TRPM3 by Phosphoinositides

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Poster N^{O}85

TRPM3 belongs to the Melastatin family of Transient Receptor Potential (TRP) ion channels. It is a Ca2+ permeable outwardly rectifying nonselective cation channel. TRPM3 is expressed in sensory neurons, brain, pancreas, kidney and vascular smooth muscle. In sensory dorsal root ganglion (DRG) neurons it was shown to function as a sensor for noxious heat. TRPM3-/- mice have decreased ability to detect noxious heat. In DRG neurons as well as pancreatic beta cells, the neurosteroid pregnenolone sulfate has been shown to activate TRPM3.

Many members of the TRP family are regulated by phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Thus we decided to test if TRPM3 is also regulated by PtdIns(4,5)P2. PtdIns(4,5)P2 is predominantly present at the cytoplasmic face of the plasma membrane and it is an important signaling molecule. In the current study we modulated the levels of PtdIns(4,5)P2 in Xenopus laevis oocytes and HEK cells heterologously expressing TRPM3. In inside-out patches, TRPM3 currents ran down rapidly after excision. This current could be restored by the exogenous application of water soluble diC8 PtdIns(4,5)P2 and the naturally occurring arachidonyl-stearyl (AASt) PtdIns(4,5)P2. Also, application of MgATP to excised inside-out patches reactivated the TRPM3 current. This effect of MgATP was inhibited by LY294002 at a concentration where it inhibits phosphatidylinositol 4 kinase (300 µM) but not at a concentration where it inhibits phosphatidylinositol 3 kinase (10 µM). This suggests that MgATP acted via replenishing PtdIns(4,5)P2 and PtdIns(4)P. In addition, inducing the activity of PtdIns(4,5)P2-5-phosphatases in whole-cell patch clamp experiments also decreased TRPM3 currents. Overall our data collected through excised inside-out and whole-cell patch clamp measurements suggest that TRPM3 requires PtdIns(4,5)P2 as a cofactor.

Telomerase and its role in modulating the cellular response to oxidative stress: a matter of location

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Poster N^{O}86

Telomerase is a ribonucleoprotein primarily responsible for telomere maintenance that is a common target in cancer therapy. Since the catalytic component of telomerase (TERT) is also mitochondrial, it is anticipated that inhibition of telomerase will not only affect telomere biology but also mitochondrial function. Consistent with this view, TERT extinction in a cancer-prone mouse model led initially to slow tumor growth, which was associated to short telomeres, and to mitochondrial dysfunction. Interestingly, while reinstatement of telomere maintenance by recombination (ALT) allowed for the re-emergence of resistant tumors, it did not completely alleviate the mitochondria dysfunction. These results indicate that, in terms of mitochondria, TERT and ALT are not the same. Furthermore, they lead to the hypothesis that lack of telomerase specifically in mitochondria can set in motion a mitochondrially-driven signaling cascade that allow cells to adapt to and cope with the lack of mitochondrial telomerase. Here we show using normal cells expressing wild type or a mutant TERT that is active in the nucleus but not able to enter mitochondria that this signaling cascade exists and is TERT and mitochondrial ROS-driven, that it involves AMP-activated protein kinase (AMPK) and autophagy, and that it leads to resistance to exogenous oxidative stress. Furthermore, our preliminary data also show that in human tumors the subcellular localization of TERT is heterogeneous, indicating that effective anti-telomerase therapies not only should consider the type of cancer and of chemotherapeutic agent but also the benefits/risks associated to inhibition of mitochondrial telomerase.

Effects of Gamma 6 Subunit on Cav1.2 Calcium Channel Function

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Poster N^{O}87

Cellular calcium signaling is responsible for varied cell type dependent responses. Cav1.2 is a L-type, high voltage activated calcium channel. Cav1.2 is expressed in heart, endocrine cells and brain. Along with the a1c subunit Cav1.2 always contains a beta subunit and may contain an alpha-2-delta or gamma auxiliary subunit. The most homologous of eight identified gamma genes are gamma 1 and gamma 6. This study is mainly focused on gamma 6's effect on the Cav1.2 channel function and how its effect differs from that of gamma 1.
We have found that gamma 6 reduces Cav1.2 currents when compared to cells expressing Cav1.2 without gamma 6. However, a version of gamma 6 missing its first 30 N-terminal amino acids allows for Cav1.2 currents similar to cells expressing Cav1.2 without gamma 6. Unlike gamma 1, this N-truncated gamma 6 does not cause voltage dependent inactivation of Cav1.2. Our modeling of gamma 1 and gamma 6 indicates that gamma 6's N-terminus contains a short alpha helix. Our modeling also suggests that the first extracellular loops of gamma 1 and gamma 6 may be somewhat structurally divergent, responsible for the difference in inactivation properties. Structural alignment of our gamma models shows several structurally identical residues that may be involved in binding to the a1c subunit. This information may be useful in developing molecules that prevent gamma 6 from binding to a1c, increasing L-type calcium currents in cells.

The role of soluble guanylyl cyclase (sGC) in cGMP-independent cardioprotection

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Poster N^{O}88

S-nitrosation (SNO) is a protein post-translational modification characterized by a nitric oxide (NO) moiety addition to a free thiol of cysteine. Protein can be S-nitrosated by S-nitrosating reagent (e.g. S-nitrosocysteine, CSNO) or through transnitrosation, which is the transfer of a NO moiety between two proteins. Soluble guanylyl cyclase (sGC) is the main NO receptor, producing cGMP when it is stimulated by NO. cGMP in turn activates protein kinase G (PKG). Recently, the lab has shown that sGC overexpression increases the SNO level of specific proteins compared to GFP overexpression, suggesting that sGC could act as a "transnitrosylase". A few proteins are proposed to mediate transnitrosation, including thioredoxin and PDI. By mass spectrometry (MS) analysis, we identified an increase in SNO level of several intercalated disc (ID) and cytoskeletal proteins in CSNO-treated neonatal cardiomyocytes infected with sGC.
sGC has to be a heterodimer, composed of α and β subunits, to produce cGMP. Yet, α1 and β1 subunits are in different cellular compartments in neonatal cardiomyocytes and adult heart tissues. β1 subunit is found in nuclear envelop, cytosol and cell membrane, especially the ID; however, α1 is predominantly in the cytosol. Interestingly, SNO level of several MS-identified targets is increased by overexpressing a single α1 or β1 subunit, suggesting a cGMP-independent process. Protein-SNO is proposed to take part in cardioprotection against ischemia/reperfusion injury. Hence, we hypothesize that single subunit of sGC, α or β, can function separately to S-nitrosate its targets; therefore, exerting its cardioprotective role independently of the cGMP/PKG pathway.

Immunization of Mice With Muscle Specific Tyrosine Kinase Alters Motor Nerve Function and Morphology

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Poster N^{O}89

Autoantibodies to muscle specific tyrosine kinase (MuSK) and the acetylcholine receptor (AChR) produce distinct forms of myasthenia gravis (MG). For example, MuSK-MG patients do not exhibit significant loss of AChRs and are refractory to anticholinesterase drug therapy. Furthermore, studies of experimental models of MuSK-MG raise the possibility that loss of endplate AChRs due to MuSK autoantibodies or decline of motor nerve function is the primary cause of MuSK-MG pathophysiology. To enhance understanding of MuSK-MG pathophysiology we studied C57B6 female mice injected with 40 µg of rat MuSK ectodomain emulsified in 100 µl of equal volumes of PBS and 50% complete Freund's adjuvant, once a month for 3 months. While all immunized mice produced MuSK antibodies, the neuromuscular symptoms of MG varied. Since in vivo plethysmography indicated that respiratory muscle function was severely altered, we measured force production for phrenic nerve – diaphragm muscle preparations. Twitch and tetanic responses to phrenic nerve stimulation were significantly less than control for 40% of MuSK injected mice. These affected MuSK MG mice provided nerve-muscle preparations for electrophysiologic, morphologic, and biochemical study. Endplate current (EPC) measurements indicated that neuromuscular transmission declined progressively in three stages. In stage 1, EPC quantal content was significantly less than control although, failures of EPC initiation were never observed. During stage 2, reduced quantal content accompanied an intermittent failure of EPC initiation that was associated with failure of action potential propagation into the motor nerve terminal. During stage 3, stimulus-evoked EPCs were absent at neuromuscular junctions which produced miniature EPCs. Failure of impulse propagation into and evoked transmitter release from the motor nerve terminal during stages 2 and 3 is attributed to motor nerve terminal neurofilament-positive swellings as well as abnormal preterminal branching. Along with the decline of stimulus-evoked transmitter release, probability of release of vesicles, number of release sites as well as the functional store of quanta declined during MuSK-MG. A prolonged decay of EPCs for MuSK affected preparations was due to a decline of acetylcholine esterase activity and not to expression of the embryonic AChR. Postsynaptically, there was also a 20% decline of AChR labeled endplate area. However, the level of AChR decline was insufficient to account for the severe depression of neuromuscular transmission observed. These data support the hypothesis that motor nerve abnormalities are the key determinants of reduced neuromuscular transmission during MuSK-MG and provide novel insight into the pathophysiology of the disease.

Role of the impaired mitophagy in the development of dystrophic cardiomyopathy

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Poster N^{O}90

DMD is a developmental muscle wasting disease affecting approximately 1 in 3200 boys. DMD patient has mutation on dystrophin gene and lack of functional dystrophin. 90 % of patients develop cardiomyopathy, less than 20 % can survive. The mechanism of cardiomyopathy development in DMD patients is still unclear. Some evidences suggested damaged mitochondria contribute to cardiomyocytes dysfuntion and death. PTEN-induced putative kinase 1 (PINK1) is a critical protein in mitochondrial quality control process which limits mitochondrial damage through mitochondial autophagy (mitophagy). Deficiency or mutation of either PINK1 or its dowstream protein Parkin impairs the mitophagy and contributes to cell death in the neurodegeneration diseases such as Alzheimer's and Parkinson's disease. Recently a group reported that the heart of PINK1 knock out mice exhibited reduced contractility and more apoptosis. Our lab found that PNIK1 protein level decreased in the heart of mdx mice (DMD animal modal). We assume that due to deficiency of PINK1, impaired mitophagy contributes to deterioration in dystrophic cardiomyocytes.

Modulation of endothelial hyperpermeability deactivation by ERM proteins

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Poster N^{O}91

Endothelial hyperpermeability is a hallmark of inflammation. Endothelial barrier function is restored after a period of hyperpermeability, but the mechanisms that deactivate hyperpermeability are largely unknown. Exchange protein activated by cAMP (Epac) is emerging as a possible deactivating/restorative factor in the endothelium. Epac localization, which is modulated by Ezrin/Radixin/Moesin (ERM) proteins, may determine its function due to cellular compartmentalization of cAMP signaling. We report preliminary results testing the hypothesis that ERM proteins facilitate deactivation of hyperpermeability by localizing Epac to the plasma membrane of endothelial cells. We demonstrated association between Epac and ERM proteins in human microvascular endothelial cells (HMVEC) by co-immunoprecipitation. Stimulation of HMVEC with platelet-activating factor (PAF) or vascular endothelial growth factor (VEGF) induced ERM protein activation. Inhibition of endothelial nitric oxide synthase (eNOS) and of PKC decreased PAF-induced ERM phosphorylation. Depletion of Radixin and Moesin by siRNA increased baseline HMVEC monolayer permeability to macromolecules, as well as PAF-induced permeability. We conclude that inflammatory stimuli activate ERM proteins in a NO- and PKC-dependent fashion. We propose that deactivation of PAF-stimulated hyperpermeability is implemented in a time-orchestrated manner, by PAF-induced activation of ERM proteins, which locate Epac to the plasma membrane promoting its interactions with endothelial junctional proteins. (Supported by NIH grants 5RO1 HL070634 & 5RO1 HL088479).


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