32nd Annual Graduate Research Conference

32nd Annual UMB Graduate Research Conference

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Foreword by GSA Executive Board

Welcome to the 32nd Annual Graduate Research Conference (GRC)! The Graduate Student Association of the University of Maryland, Baltimore (UMB) has been dedicated to this project since the beginning of the school year. Each year, the GRC familiarizes graduate students with preparing for scientific meetings, as well as the opportunity to present results of their ongoing research in an interdisciplinary setting to peers, faculty members, and the UMB community at large. Approximately 50 students from across the UMB campus will present their work as either poster or oral presentations at this GRC, and we would like to thank each of the presenters for their time and effort to formally communicate their achievements – we commend your hard work and devotion to your science.

This year we will continue to take an interdisciplinary approach to the conference which highlights research across fields and even schools. We hope this will continue to enrich the students' experience as well as challenge them to apply their work to a new and broader audience. This year, GRC participants also have the opportunity to be considered for two special awards. The Geriatrics and Gerontology Education and Research Program (GGEAR) will be sponsoring awards in aging research to graduate students who have either completed research or have research in progress in Social science/behavioral/clinical research or Bio-medical/basic science research in the field of aging. The Office of Commercial Ventures & Intellectual Property (CVIP), in association with the Graduate Research Conference, is delighted to announce the first annual CVIP Translational Graduate Research Award. The award is made in recognition of important translational research performed by a UMB graduate student and encompasses a wide array of disciplines with biomedical or other practical applications.

The Graduate Student Association Executive Board would like to thank everyone who has contributed to this year's conference. Specifically, we would like to acknowledge Dr. Malinda Orlin, Vice President for Academic Affairs and Dean of the UMB Graduate School; Dr. Erin Golembewski, Associate Dean of the UMB Graduate School; and all the members of the UMB Graduate School Office. Additionally, we would like to thank the faculty members who have volunteered their time to serve as judges and mentors – your dedication to the advancement of your students here today, and everyday, is greatly appreciated. Thank you to the GSA Program Representative volunteers for your dedication, energy, and initiative. Finally, we would like to acknowledge the Graduate Program in Life Sciences (GPILS) for sponsoring our keynote speaker Mr. Steve Mirsky, who we are privileged to have here today.

We hope that you enjoy your experience at this year's GRC. We have worked hard to make the day as enjoyable and informative as possible. We invite you to participate fully in this year's conference and we look forward to welcoming you back next year. It has truly been a privilege and honor to provide a colloquium for all graduate students of the UMB community to present their achievements.

<u>Graduate Student Association Executive Board</u>
Michael Morgan, President
Jim Kaspar, Vice-President
Shannon O'Connor, Treasurer
Anna Seekatz, Secretary
Ross Cardarelli, Graduate Council Representative

32nd Annual Graduate Research

Conference Keynote Speaker

MR. STEVE MIRSKY
PODCAST EDITOR, SCIENTIFIC AMERICAN

Steve Mirsky has written the allegedly humorous Anti Gravity column for Scientific American since 1995 and is a member of the magazine's board of editors. A collection of his columns, cleverly called Anti Gravity, was published by The Lyons Press in 2008. He has contributed to numerous publications and broadcast outlets, including Audubon; Wildlife Conservation; National Wildlife; Earth; Longevity; The Humanist; Men's Fitness; American Health; Technology Review; the Howard Hughes Medical Institute Bulletin; Astronomy; Eating Well; American Airlines in-flight magazine; New York Newsday; Sea Frontiers; the children's magazines Current Science, Science World and Muse; National Public Radio; and the Medical News Network (which folded 90 minutes into his first day on the job).

Mirsky received a Master's Degree in chemistry from Cornell University in 1985, after which he was awarded a Mass Media fellowship by the American Association for the Advancement of Science to work for a summer as a science journalist at WSVN-TV in Miami. This critical event removed him from the lab, to the great relief of the American Chemical Society and the Ithaca Fire Department. He then spent a year as a morning radio host at WMCR in Oneida, NY. Upon returning to his home in New York City, he became a staff editor at Breakthrough, a newsletter concentrating on science and technology (which folded 12 days after he came on board). He then spent five years writing about basic research for publications of the Albert Einstein College of Medicine before returning to freelancing fulltime. He joined the staff of Scientific American in 1997.

Mirsky has been awarded science journalism fellowships at the Marine Biological Laboratory at Woods Hole, Massachusetts, where he studied molecular evolution, and at Columbia University. He also received a Knight Science Journalism Fellowship at the Massachusetts Institute of Technology for the 2003-2004 academic year, which he primarily spent slumming in Alan Dershowitz's criminal law class at the Harvard Law School.

In February, 2006, Mirsky developed the weekly Scientific American podcast, the magazine's foray into web-based broadcasting. He hosts the program, called Science Talk: The Weekly Podcast of Scientific American. Over 200 episodes are now on the web. Guests have included seven Nobel Laureates, a Pulitzer Prize winner, an Academy Award winner and Mirsky's dad. In September, 2006, Mirsky launched the daily Scientific American podcast, 60-Second Science, which he produces and often hosts. Over 900 episodes are now on the web. The programs were nominated for a 2007 Webby Award, recognizing the best of the internet. And it's an honor just to be nominated.

SCHEDULE OF EVENTS

32nd Annual Graduate Research Conference

Thursday, April 8, 2010

University of Maryland, Baltimore

8:00 - 9:00am	**BREAKFAST & REGISTRATION**
9:00am - 12:30pm	**POSTER PRESENTATIONS**
9:30 - 11:00am	Basic Sciences Poster Sessions A, B (Room 349)
11:00 - 12:30pm	Basic Sciences Poster Sessions C, D (Room 349)
9:00am - 12:30pm	**ORAL PRESENTATIONS**
9:30 - 11:00am	Social Sciences Oral Session A (Room 353)
9:00 - 10:30am	Basic Sciences Oral Session E (Room 203)
10:30- 12:30pm	Basic Sciences Oral Session F (Room 223)
12:30 - 2:00pm	**LUNCH & KEYNOTE ADDRESS** (Ballroom)
	Steve Mirsky
	Podcast Editor, Scientific American
2:00pm	**AWARDS** (Ballroom)

Abstracts

1. DISRUPTING NEUROGENESIS AT E19/20 IMPAIRS MORRIS WATER MAZE PERFORMANCE AND ATTENUATES HIPPOCAMPAL-MPFC LTP IN ADULT MALE RATS

P. L. Brown, S. Stockman, R. McFarland, G. I. Elmer, P. D. Shepard, M. W. Vogel

Poster Presentation; Room 349; Basic Science A

Disruption of embryonic neurogenesis has recently been proposed as a means of modeling in animals some of the neuropathological and behavioral deficits seen in schizophrenia. An anti-mitotic agent, cytosine arabinoside (AraC), applied at various times during gestation causes subtle disruptions in neurodevelopment that lead to significant perturbations in the adult animal. Recent work has shown that AraC application to rats on embryonic day 19 and 20 (E19/20) disrupts pre-pulse inhibition (PPI) and hippocampal neuroanatomy (Elmer et al., 2004). In the current study, we investigated possible AraC-induced impairment of hippocampal-cortical circuitry using two methods: the Morris Water Maze (MWM) and long-term potentiation (LTP) in the hippocampal-prefrontal cortex (PFC) circuit. Over four days of acquisition training, saline treated rats were readily able to learn the hippocampal and PFC dependent learning MWM task, exhibiting an average latency of about 20s in finding the platform by the end of training. In contrast, AraC treated rats showed longer latencies throughout each day of training and had an average latency of about 60s by the end of training. Latency to find a visible platform did not differ between groups. Following MWM testing, long-term potentiation (LTP) in the hippocampocortical pathway was assessed in the same rats. Evoked field potentials were recorded in the medial PFC following stimulation of CA1/subiculum in the ventral hippocampus. After a 30 min control period, two high frequency trains were delivered, followed by another 60 min of evoked potential recording. In saline-treated rats, tetanic stimulation led to an approximate 50% increase in field potential amplitude that lasted the length of the experiment. In contrast, AraC treated rats showed an attenuated potentiation of approximately 25% above baseline without attenuation in the duration. Given that the MWM is hippocampal and PFC dependent, and that LTP is believed to mediate learning and memory, we predicted a significant correlation between MWM performance and LTP. In fact, the degree of LTP correlated well with behavioral performance regardless of the group designation. These data, along with the previously demonstrated neuroanatomical and PPI deficits, support the proposition that transient disruption of neurogenesis using AraC is a potentially useful animal model for elucidating the neurobiological basis of cognitive deficits in schizophrenia.

2. ABASIC DNA IS A POTENT INHIBITOR OF THYMINE DNA GLYCOSYLASE (TDG)

Megan E. Fitzgerald and Alexander C. Drohat

Poster Presentation; Room 349; Basic Science A

DNA glycosylases initiate the base excision repair (BER) pathway by removing damaged bases, producing an abasic (AP) site in the DNA. AP endonuclease I (APE1) then displaces the glycosylase and nicks the phosphodiester as limiting its enzymatic turnover. APE1 stimulates the turnover of TDG TDG binds tightly to AP DNA with specificity for CpG sites. Like other glycosylases as well as other lesions backbone, and the correct nucleotide is restored by other BER enzymes. Thymine DNA glycosylase (TDG) is one of the human enzymes that recognizes and initiates repair of GT mismatches as it does for other DNA glycosylases. This suggests the first steps of BER are coordinated to avoid release of toxic AP sites yet the detailed mechanism of the APE1 effect remains unresolved. Using burst kinetics

and steady-state kinetics experiments we have observed very slow turnover (kcat) and a dramatic stimulation of TDG turnover by APE1. Previous studies by us and others indicate APE1 actively disrupts the TDG product complex as opposed to simply depleting the concentration of AP-DNA. To further elucidate this mechanism we have performed off-rate studies for both substrate and the AP-DNA product. The product off-rate is faster than expected and thus not fully rate-limiting for all substrates. Off-rates for substrates showed a trend that can be related to KD and kcat values G"FU and G"U have a much slower off-rate than G"T substrates. These differences in off-rates and the inverse correlation to kcat lead us to hypothesize that kcat is limited by product inhibition in addition to slow product release. Using inhibition and binding experiments we have found a significant inhibition of TDG by AP DNA for all substrates with the greatest affect observed for G"T reactions. The 1800-fold difference in kcat for G"T vs. G"FU substrates was first thought to reflect similar differences in koff but measured koff appear to be the same. Thus this difference in kcat indicates a difference in inhibition by AP-DNA for the substrates. We propose AP-DNA is a tight slow-binding inhibitor of TDG affecting reactions most severely where commitment to catalysis is low i.e. for G"T substrates. This suggests that APE1 most likely increases TDG s product off-rate as well as depletes and/or sequesters AP-DNA in order to stimulate turnover of TDG.

3. Risk of Childhood Exposure to Pesticides Related to Athletic Field Maintenance Practices: A Survey of Frequencies and Factors

Robyn Gilden

Poster Presentation; Room 349; Basic Science A

The US Environmental Protection Agency (2008) defines a pesticide as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest", whether insect, plant, fungus, rodent, or bacteria. The usual targets of many pesticides are the pests' nervous or reproductive system, although they may act in other ways in any organism, including humans. Pesticides are ubiquitous in our environment and are leading to increasing body burden (CDC, 2005). We are exposed to pesticides at home, work, and in the community through consuming food and water, breathing air, and through contact with soils or on surfaces. There are much data, both animal and human, demonstrating that pesticide exposures have acute and chronic health effects, including neurologic and neurodevelopmental, reproductive and endocrine, immune system, and cancer but most of the toxicological data related to health effects from exposure are based on studies focusing on one chemical via one route. There has been little exploration taking into consideration the many possible combinations of chemicals, routes of exposures, and exposed individuals that actually occur in reality, such as those exposures that may occur on athletic fields. This study assessed potential exposure to pesticides based on maintenance practices on athletic fields, including use of pesticides and the factors that may be related to likelihood of usage, including field location, field ownership/oversight, and field characteristics. Randomly selected athletic fields (N=101) in the six (6) jurisdictions of Central Maryland participated, including public and private schools, colleges and universities and public fields. A survey was emailed or administered over the phone to the responsible field manager assessing ownership, field conditions, and maintenance practices, including the use and types of pesticides. Sixty-six fields (65.3%) out of 101 reported using some form of pesticides, mainly herbicides (n=58, 57.4%). Pesticide use did vary by density with urban and suburban fields being less likely to use pesticides than rural fields. Combined preventive practices (soil testing,

aerating, and overseeding) was also a significant predictor of pesticide use with conducting preventive practices being associated with pesticide use. This is possibly an indication that the better monitoring indicating a larger budget available to purchase pesticides as well. Results from this study can be used to support drafting model policy language. Such changes in pesticide use policy will improve health of workers, sports participants, and observers thereby reducing health care costs and missed work and school days. Findings from this study can also be used to improve education of local officials, field maintenance personnel and general public on health effects related to pesticides and non-toxic management of lawns and playing fields.

4. MEASURING INTRA-CELLULAR AND INTRA-MITOCHONDRIAL ZINC CONCENTRATIONS FOLLOWING HYPOXIA/HYPOGLYCEMIA WITH AN EXPRESSIBLE RATIOMETRIC FLUORESCENCE BIOSENSOR

Bryan McCranor, Linda Bambrick, Rebecca Bozym, Gary Fiskum, Richard Thompson

Poster Presentation; Room 349; Basic Science A

Zinc is a "trace" metal necessary for proper cellular function. Studies have shown that the intra- and extra-cellular concentrations of labile zinc increase dramatically in models of cerebral ischemia (1, 2). Substantial evidence indicates that mitochondrial dysfunction plays a significant role in neuronal death following ischemia. Both mitochondrial dysfunction and increased intracellular zinc concentrations have been associated with increased reactive oxygen species (ROS) production and ultimately apoptosis (3, 4). We modified our fluorescent zinc biosensor (5) to be selectively expressed in the mitochondria of PC12 cells, enabling us to ratiometrically image the intra-mitochondrial concentration of labile zinc even at resting (picomolar) levels. We used this expressible biosensor and our previous sensor in cells which have undergone oxygen/glucose deprivation (OGD). Our initial results indicate that the concentration of labile, intra-mitochondrial zinc may not increase to the degree that we observed in the cytoplasm during hypoxic/hypoglycemic conditions, and may be lower than the concentrations observed in cells in more physiological conditions. 1. Tonder, N., et al. (1990) Neuroscience Letters 109, 247-252. 2. Frederickson, C.J., et al. (2006) Experimental Neurology 198, 285 - 293. 3. Weiss, J. H., Sensi, S. L. & Koh, J.-y. (2000) Trends in Pharmacological Sciences 21, 395 - 401. 4. Jiang, D., et al. (2001) Journal of Biological Chemistry 276, 47524 - 47529. 5. Bozym, R. A., et al. (2006) ACS Chemical Biology 1, 103; 111

5. THE ADAPTABILITY OF INTERLIMB COORDINATION DURING WALKING IN INDIVIDUALS WITH POST-STROKE HEMIPARESIS AND NON-DISABLED ADULTS.

DN Savin, Tseng, S-C, Whitall J, Morton SM

Poster Presentation; Room 349; Basic Science A

Appropriate interlimb coordination is essential for normal human locomotion. Patterns of coordination between the legs must also be adaptable to accommodate changes in the environment, the body or the behavioral goal. Because bipedal gait is inherently less stable than quadruped, humans may require greater supraspinal control of gait than lower mammals. Presently, little is known about how stroke and resulting hemiparesis affects adaptive mechanisms influencing interlimb coordination during locomotion. To investigate this, we tested locomotor adaptations made by 14 individuals with chronic unilateral stroke and hemiparesis and 12 age- and gender-matched non-disabled adults. The paradigm involved walking on a treadmill in three successive conditions: Baseline, Adaptation and Post-adaptation. Subjects with stroke walked at a self-selected speed, which controls

matched. During Adaptation, a mechanical perturbation was introduced that resisted the forward movement of one of the legs. The perturbation was then removed in the Post-adaptation condition. Outcome measures to quantify adaptation of interlimb coordination were step length and single limb support duration. All subjects adapted interlimb coordination to the perturbation, showing bilateral changes in step length and single limb support duration that sustained interlimb coordination and walking symmetry. Step length and single limb support duration initially decreased in the perturbed leg and increased in the un-perturbed leg, but both gradually returned to near-baseline values. Storage of the adaptation was demonstrated in both groups by the presence of negative aftereffects, i.e. changes in the walking parameters in the opposite direction during Post-adaptation. Our results indicate that individuals with stroke and hemiparesis are able to adapt interlimb coordination similar to controls. We conclude that the ability to adapt interlimb coordination patterns during walking may not be affected by stroke involving brain centers controlling descending motor commands to the legs.

6. Parameter Optimization and Analysis of Amino Acid Side Chains Based on MM, QM, and Experimental Data

Xiao Zhu, Pedro Lopes, Jihyun Shim, Alexander D. MacKerell

Poster Presentation; Room 349; Basic Science A

Amino acid side chain flexibility is an important property that influences the stability of the folded state of proteins. In molecular mechanics, the conformational properties of sidechains is largely dictated by torsional parameters. In this study, we analyze the conformational properties of sidechains via quantum mechanical calculations. One and two- dimensional chi energy surfaces were performed on dipeptides representative of the amino acids. Analysis was performed for relevant peptide backbone conformations corresponding to the alpha helical (alpha R), beta stranded (extended) and alpha L conformations. QM optimizations were performed at the MP2/6-31G(d) or MP2/6-31+G(d) levels followed by single point calculations with RIMP2/cc-pVTZ. The resulting energy surfaces are indicative of the conformational properties of the different amino acid sidechains and the data is of utility as target data for force field optimization.

7. Functional Characterization of Iron-Substituted Neural Zinc Finger Factor 1

Angelique N. Besold, Seung Jae Lee, Niall Lue Sue, Holly J. Cymet, Sarah L. J. Michel

Poster Presentation; Room 349; Basic Science B

Zinc finger proteins are transcription factors that are ubiquitous in eukaryotes. Neural zinc finger-factor 1 (NZF-1) is a non-classical zinc finger protein that is involved in neuronal development. The unique CCHHC zinc binding domains of NZF-1 are involved in the recognition of the β-RARE promoter element in the β-retinoic acid receptor gene. Zinc naturally binds to this domain, but it can be speculated that iron can bind instead. Iron is very prevalent in the nervous system, as it is important for proper functioning. Misregulation of iron, a highly redox active metal, can have serious consequences. The iron binding affinity for a double domain (NZF-1-DF) fragment of NZF-1 was examined. Both Fe(II) and Fe(III) were shown to bind this fragment of NZF-1. Fe(III) bound NZF-1-DF with micromolar affinity. Fluorescence anisotropy was used to determine the DNA binding affinity of the iron bound NZF-1-DF. The resulting binding affinity for both Fe(II) and Fe(III) bound NZF-1-DF was comparable to that of the Zn(II) bound NZF-1-DF peptide. This could suggest possible localized

oxidative damage to genes that are regulated by NZF-1.

8. Purification and characterization of MBD4

Timothy Howes, Michael Morgan, and Alexander C. Drohat

Poster Presentation; Room 349; Basic Science B

Methyl CpG Binding Domain 4 (MBD4), also known as MED1, is a DNA glycosylase enzyme that initiates base excision repair of T-G mispairs at CpG sites. It maintains genomic stability by cleaving the glycosidic (base-sugar) bond of thymines arising from deamination of 5-methyl-cytosine. Disruption of MBD4 by downregulation or mutation, has been observed in several types of cancer, including ovarian and colorectal. We are initiating studies of MBD4 to understand how it recognizes G-T mispairs and avoids acting upon the vast excess of normal DNA. Both the full length human MBD4 and catalytic domain MBD4(426-580) were overexpressed in E.coli. Purification of MBD4(426-580) was achieved via nickel affinity, ion exchange, and size exclusion chromatography. Full length MBD4 appears to be aggregating into inclusion bodies , and it also seems to be sensitive to proteolysis. We are exploring strategies to handle these complications. Additionally, growth and induction conditions for the full length enzyme were examined. The ability of MBD4 to cleave thymine as well as uracil, was measured with single turnover kinetics, and reaction progress was measured by high performance liquid chromatography. MBD4 cleaves thymine and uracil paired with guanine, at rates of 1.6min-1 and 5.1min-1 respectively.

9. Obscurin signaling through its MLCK domains

Li-Yen Hu, Aikaterini Kontrogianni-Konstantopoulos

Poster Presentation; Room 349; Basic Science B

Obscurins are giant muscle proteins implicated in myofibrillogenesis and linked to hypertrophic cardiomyopathy. They contain a tandem array of adhesion (i.e. immunoglobulin and fibronectin-III) domains as well as of signaling motifs, including an isoleucine-glutamine (IQ) repeat, a Src homology 3 (SH3) domain, a pleckstrin-homology (PH) domain, a Rho-guanine nucleotide exchange factor (Rho-GEF) domain, and two serine/threonine kinase domains, namely SK1 and SK2. Three out of the four reported obscurin isoforms that arise from complex alternative splicing of the single OBSCN gene contain single or tandem kinase domains. Using immunofluorescence combined with confocal microscopy and antibodies specific to the kinase-bearing obscurin isoforms, I found that they are present in diverse locations in the cardiac cell, including the sarcolemma, the Z/I junction and the M-band. To identify potential binding partners or substrates of SK1 and SK2 in cardiac cells, I employed the yeast two-hybrid system. Importantly, my screening demonstrated that Na+/K+-ATPase and N-cadherin, both of which are major components of adherens junctions, are potential interacting partners of SK1 and SK2, respectively. Detailed deletion analyses indicated that the extracellular domain of the β-subunit of Na+/K+-ATPase is sufficient to interact with the catalytic portion of SK1, whereas both the extracellular and intracellular regions of N-cadherin are required to interact with the catalytic portion of SK2. Currently, I am investigating the roles of SK1 and SK2 in regulating cardiac function by manipulating their expression levels through adenoviral-mediated gene delivery.

10. DIETARY SUPPLEMENTATION WITH DOCOSAHEXAENOIC ACID, BUT NOT EICOSAPENTANOIC ACID, REMODELS CARDIAC MITOCHONDRIAL PHOSPHOLIPID FATTY ACID COMPOSITION AND PREVENTS PERMEABILITY TRANSITION

Ramzi J. Khairallah, Genevieve C. Sparagna, Nishanth Khanna, Karen M. O'Shea, Gary Fiskum, Christine Des Rosiers, William C. Stanley

Poster Presentation; Room 349; Basic Science B

Treatment with the É-3 polyunsaturated fatty acids (PUFAs) docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) exerts cardioprotective effects in patients, and suppresses Ca2+-induced opening of the mitochondrial permeability transition pore (MPTP) in vitro. These effects are associated with increased DHA and EPA and lower arachidonic acid (ARA) in cardiac phospholipids. ARA is implicated in inflammation and induction of MPTP opening. While clinical studies suggest the triglyceride lowering effects of DHA and EPA are equivalent, there is growing evidence that DHA may be superior at remodeling mitochondrial phospholipids and preventing MPTP. Therefore we compared the effects of dietary supplementation with the É-3 PUFAs DHA and EPA on cardiac mitochondrial phospholipid fatty acid composition and Ca2+-induced MPTP opening. Rats were fed either a control (CTRL) low-fat chow, or a similar diet supplemented with either DHA or EPA only at 2.5% of energy intake for 8 weeks. These doses of DHA and EPA are comparable to ~5g/day in humans. Cardiac mitochondria were isolated and analyzed for Ca2+-induced MPT, respiration, and phospholipid fatty acyl composition. Both DHA and EPA enriched diets lowered circulating free fatty acids and triglycerides by approximately 40% ($p<0.05$, DHA vs CTRL and EPA vs CTRL, NS, DHA vs EPA). DHA supplementation increased DHA by 63% ($p<0.05$ vs control) and decreased ARA by 61% ($p<0.05$ vs control) in mitochondrial phospholipids, and significantly delayed MPTP opening (57% more calcium necessary to induce MPTP vs CTRL, $p<0.05$). EPA supplementation did not affect DHA, only modestly lowered ARA (-33% vs CTRL, $p<0.05$), and had no effect of MPTP opening. State 3 respiration with a variety of substrates was unaffected by dietary treatment, however DHA decreased state 4 respiration by 30% and the increased RCR by 70% with pyruvate+malate as the substrate, both in the absence and presence of oligomycin ($p<0.05$); treatment with EPA had no effect. The P:O ratio was not different among groups with any of the substrates. In summary, DHA supplementation favorably altered mitochondrial phospholipid composition and delayed MPT in cardiac mitochondria, while EPA had no effect. These effects may contribute to the protection against heart disease with É-3 PUFA supplementation, and suggest that supplementation with DHA should be superior to EPA.

11. EARLY TREATMENT WITH PGE2 AND COX-INHIBITORS AFFECT DENDRITIC BRANCHING OF CEREBELLAR PURKINJE CELLS WITH IMPLICATIONS FOR SEX DIFFERENCES IN SOCIAL AND EXPLORATORY BEHAVIOR

Jessica F. Knutson, Shannon L. Dean, Margaret M. McCarthy

Poster Presentation; Room 349; Basic Science B

Over-the-counter fever reducers such as aspirin function by inhibiting cyclooxygenase isoenzymes COX-1 and COX-2, which are important for the conversion of arachidonic acid to prostaglandin E2 (PGE2). PGE2 is a key regulator of fever following inflammation throughout the central nervous system, although its role in the cerebellum hasn't been established. Treatment with PGE2

during the second week of postnatal development decreases Purkinje cell dendritic length and spine number in the cerebellum, while COX-inhibitors, which decrease PGE2, have the opposite effect. Treatments with estradiol during the same time period have effects similar to that of PGE2. PGE2 stimulates the synthesis of the steroid hormone estradiol and co-treatment of PGE2 and the estradiol receptor antagonist ICI blocks the effects of PGE2 on Purkinje cell morphology, further suggesting estradiol acts downstream of PGE2 in the developing cerebellum. Normally, males show greater social play behavior than females. When treated with the COX-inhibitor nimesulide, males show reduced social play behavior, increased exploratory behavior, and a heightened somatosensory threshold. Cerebellar pathology has been associated with complex disease syndromes such as autism and schizophrenia: inflammation early in life is a risk factor in both diseases and these results suggest prostaglandins may be a contributor to that risk.

12. Determinants of Persistent Medication Use among Medicare Beneficiaries with Diabetes

Jennifer Lloyd, Bruce Stuart, J. Samantha Shoemaker, Thomas Shaffer

Poster Presentation; Room 349; Basic Science B

Persistent use of medications such as older oral antidiabetic agents, ACE inhibitors, angiotensin II receptor blockers (ARBs), and statins among diabetic patients has been shown to result in lower Medicare costs and reduced risk of hospitalization. Factors associated with persistency of medication use are unknown. This study aims to predict persistency in medication use over a 3 year study period for users of older and newer oral antidiabetic agents, ACE-Is and ARBs, as well as statins. Hypothesized predictors include disease management behaviors (taking a diabetic self-management class, testing blood sugar, diet, exercise, aspirin use, checking feet sores), disease knowledge (good, some, and poor), medication management (use of older and new oral antidiabetic agents, ACE-Is and ARBs, statins, insulin, and other lipid lowering drugs), as well as other demographic covariates. Using data from the Medicare Current Beneficiary Survey (MCBS) from 1997 to 2005, six cohorts of Medicare beneficiaries with diabetes were each followed over 3 years (N=2,187). Preliminary multivariate regression results show that persistent medication management (with the exception of insulin and other lipid lowering drugs) was positively associated with persistent use of older and newer antidiabetic agents, ACE-Is and ARBs, as well as statins ($p<0.05$). Of the 8 disease management and knowledge variables, testing blood sugar was associated with older antidiabetic agents and statins, while exercise was associated with these former drugs as well as with ACE-Is and ARBs ($p<0.05$). Increasing persistency of medication management for diabetes may have important policy implications for drug coverage and the Medicare program.

13. Biphasic Effect of Reactive Oxygen Species on Skeletal Muscle Sarcolemmal Ca2+ Influx

George G. Rodney, Luke P. Michaelson, Guoli Shi, Christopher W. Ward

Poster Presentation; Room 349; Basic Science B

In striated muscle, sarcolemmal Ca2+ influx (SCI) serves to maintain the store of Ca2+ within the sarcoplasmic reticulum during repetitive contractions. However, dysregulated SCI may alter the Ca2+ homeostasis implicated in the pathogenic progression of Duchenne Muscular Dystrophy (DMD). Despite the importance of SCI, we have limited insight into the manner SCI affects health and disease.

This research investigates the potential role of oxidative stress on SCI in wild type (WT) and in mdx (murine DMD model) myofibers. WT and mdx myofibers were loaded with the Ca2+ indicator dye Fura-2 AM and were subjected to the Mn2+ quench protocol. In short, the rate of fluorescence quench by the Mn2+ ringer solution served as a surrogate for SCI rate. WT and mdx myofibers were incubated with increasing concentrations of the oxidant H2O2. Additional WT and mdx groups were incubated in reduced glutathione (GSHEE, 5mM) followed by H2O2 perfusion. SCI was evaluated during long K+ induced depolarization within each condition and genotype. The findings indicate WT myofibers perfused with 10uM [H2O2] exhibited a 37% increase in depolarization induced SCI when compared to control WT fibers not perfused with [H2O2]. Unexpectedly, pre-treatment with GSHEE in WT myofibers without H2O2 perfusion also increased depolarization induced SCI by 31% when compared to control WT myofibers. In mdx myofibers, perfusing 10uM [H2O2] decreased depolarization induced SCI by 29% when compared to control mdx myofibers not treated with H2O2. Additionally, GSHEE incubation of mdx myofibers not perfused with H2O2 decreased depolarization induced SCI by 31% compared to the control mdx myofibers. These data reveal that pre-incubation of mdx fibers with GSHEE decreased SCI back toward wild-type, suggesting that SCI in mdx myofibers may be a potential therapeutic target via redox modulation. Ongoing studies are addressing the role of ROS production during strenuous exercise as a dynamic regulator of SCI in health and disease.

14. OVEREXPRESSION OF MATRIPTASE WITHOUT SUFFICIENT HAI-1 OPPOSITION MAY CONTRIBUTE TO THE MALIGNANCY OF AGGRESSIVE B-CELL LYMPHOMAS THROUGH INITIATION OF PROTEASE CASCADE AND ACTIVATION OF GROWTH FACTOR

Feng-Pai Chou, Ya-Wen Chen, Xianfeng Frank Zhao, Ronald B. Gartehaus, Michael Johnson and Chen-Yong Lin

Poster Presentation; Room 349; Basic Science C

Matriptase, a membrane-associated serine protease, is widely expressed by epithelial and carcinoma cells in which the activity of the protease is tightly controlled by hepatocyte growth factor activator inhibitor-1 (HAI-1). A partial lessening of the suppression of matriptase activity by HAI-1 converts this physiological protease into an oncogenic protease. In the current studies, matriptase was detected at very high levels in the absence of balanced expression of HAI-1 in aggressive human B-cell lymphomas. In contrast, the protease was not detected in several indolent B-cell lymphomas and hyperactive B-cells. In the virtual absence of HAI-1, the lymphoma cells are able to produce and maintain levels of active matriptase, in contrast to the situation in epithelial and carcinoma cells, where active matriptase is rapidly converted to enzymatically inactive matriptase-HAI-1 complexes. Active matriptase can activate urokinase-type plasminogen activator (uPA) to initiate a pericellular proteolysis cascade and activate hepatocyte growth factor (HGF) which plays an important role in lymphoma. These studies suggest that enhanced matriptase activity resulting from expression in the absence of significant HAI-1 expression may result in deregulated pericellular proteolysis which may contribute to the aggressiveness of some human B-cell lymphomas.

15. PHARMACOLOGIC ACTIVATION OF THE NRF2/ARE PATHWAY OF ANTIOXIDANT GENE EXPRESSION INHIBITS MITOCHONDRIAL PERMEABILITY TRANSITION AND PROVIDES NEUROPROTECTION FOLLOWING CARDIAC ARREST

T. Greco, Rosenthal, R.E., Hazelton, J., Balan, I., Racz, J., Cotto-Cumba, C., Fiskum, G.

Poster Presentation; Room 349; Basic Science C

Oxidative stress contributes to the pathophysiology of both acute and chronic neurodegenerative disorders. Mitochondrial oxidative stress can lead to both necrotic and apoptotic cell death as a consequence of metabolic failure and release of pro-apoptotic proteins. One effect of oxidative stress on mitochondria is promotion of the calcium-dependent opening of the inner membrane permeability transition pore (PTP), which depolarizes the membrane and inhibits ATP synthesis. Pharmacologic activation of the Nrf2/ARE pathway of anti-oxidant gene expression by agents such as sulforaphane provides neuroprotection both in-vitro and in-vivo but little is known about the effects of this pathway on mitochondrial responses to stress. This study tested two hypotheses: 1. administration of sulforaphane either in vitro or in vivo increases the resistance of neural cell mitochondria to PTP opening caused by oxidative stress. 2. Post-reatment of animals with SFP following cardiac arrest (CA) reduces hippocampal neuronal death and improves neurologic outcome. Sulforaphane (SFP) or DMSO vehicle was administered to rats ip at 10mg/kg 48 hr prior to isolation of brain mitochondria. PC12 cells were exposed to 1μM SFP or DMSO vehicle for 24 hr. Fluorescent based measurements of mitochondrial calcium uptake and subsequent release induced by the oxidant tert-butyl hydroperoxide (tBOOH) were performed on isolated brain mitochondria and digitonin permeabilized PC12 cells. Autofluorescence of NAD(P)H was monitored simultaneously. Anesthetized beagles were subjected to 10 min CA arrest followed by resuscitation and 24 hr critical care. At 30 min reperfusion, dogs received either 1 mg/kg SFP iv or DMSO. At 23 hr animals were awakened and a neurologic deficit test performed. Animals were euthanized at 24 hr and dying hippocampal CA1 neurons were quantified using stereology. SFP treatment substantially reduced the rate of cyclosporin A-sensitive calcium release induced by tBOOH with both brain mitochondria and permeabilized PC-12 cells. SFP attenuated NAD(P)H oxidation in brain mitochondria but not PC-12 cells. Neurologic deficit scores were lower for animals treated with SFP. SFP also reduced the percentage of dying neurons. We conclude that SFP administration after CA reduces hippocampal neuronal death and improves short-term neurologic outcome. As PTP opening is implicated as a cause of neuronal death following ischemia/reperfusion, effects of SFP on the resistance of mitochondrial PTP opening may contribute to the neuroprotective properties of SFP.

16. RECOMBINANT EXPRESSION OF LEE-ENCODED REGULATOR (LER) FROM ENTEROHEMORRHAGIC ESCHERICHIA COLI FOR OLIGOMERIC CHARACTERIZATION

Jonathan A. Levine, Anne-Marie Hansen, and James B. Kaper

Poster Presentation; Room 349; Basic Science C

Diarrheagenic Escherichia coli (E. coli) are a major cause of infant mortality worldwide. Enterohemorrhagic E. coli (EHEC) has been of particular concern in recent years because of outbreaks caused by contaminated beef and produce. The Locus of Enterocyte Effacement (LEE) - Encoded Regulator (Ler) from EHEC is the principal regulator of the LEE pathogenicty island that contains at least 5 operons, encoding

virulence factors including an adhesin, translocator, and secreted effector proteins. The function of Ler, as a positive transcriptional modulator, directly correlates with its abilities to form an oligomer. The coiled-coil region in the Ler N-terminus is responsible for oligomerization. It has been demonstrated that Ler recognizes DNA structural motifs rather than specific nucleotide sequences. Thus the mechanism of DNA recognition by LER could be elucidated by defining its oligomeric domain, state, and structure. Using mass spectrometry and affinity chromatography, we have identified the native Ler start codon. To define the oligomerization domain we employ partial tryptic digests of purified Ler. Determination of Ler s oligomeric state was be examined by size exclusion chromatography. Preliminary data suggests a higher order oligomeric state.

17. FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) AS A TECHNIQUE TO STUDY INTERACTIONS OF SMALL-MOLECULE INHIBITORS WITH MAP KINASE ERK2

Kerrick Nevels, Paul Shapiro, and Peter Butko

Poster Presentation; Room 349; Basic Science C

The extracellular-signal-regulated kinase (ERK) proteins belong to the mitogen-activated protein kinase family. They participate in several important cell-signaling pathways. Unregulated, ERK2 is thought to mediate cell proliferation in many types of cancer. But because ERK also functions in many normal cell activities, inhibition selectivity is necessary for its use in cancer therapy: one needs to block only phosphorylation of those substrates that are involved in cell proliferation, while leaving phosphorylation of other substrates unaffected. Novel small-molecule compounds, which inhibit phosphorylation of selected substrates but do not compete with ATP for its binding site, have been previously identified by computer-aided drug design (CADD) and molecular-dynamics docking algorithms. Herein, we spectroscopically characterized selected compounds, and show that they can serve as fluorescence resonance energy transfer (FRET) acceptors for tryptophan: we determined the values of spectral overlap J between the compounds' absorption and ERK fluorescence, and of the Forster distance R0 for the compound/tryptophan pairs. We then used FRET to determine average distances between the bound compounds and the group of three tryptophans in ERK2, thus validating the CADD predictions of the compounds' binding sites. The problem of multiple but closely located donors (tryptophans) that pass the energy to the single acceptor (the bound compound) is discussed, as is the possibility of utilizing FRET for mapping the inhibitor-binding sites on other kinases. The novel FRET acceptors for tryptophan can conceivably be employed in studies of other protein/ligand systems with suitable spectroscopic properties.

18. NOVEL SIGNALING INTERACTIONS BETWEEN PROTEINASE-ACTIVATED RECEPTOR 2 AND TOLL-LIKE RECEPTORS IN VITRO AND IN VIVO

Quan M. Nhu*, Prasad Rallabhandi, Kari Ann Shirey, John Teijaro, Donna Farber, Sarah Netzel-Arnett, Toni Antalis, Alessio Fasano, Stefanie Vogel

Poster Presentation; Room 349; Basic Science C

Toll-like receptors (TLRs) and proteinase-activated receptors (PARs) function as innate immune biosensors in mucosal epithelial cells (ECs). We previously reported the functional and physical interactions between TLR4 and PAR2, and more recently, between PAR2 and the TLR adapter, TRIF, and established a novel paradigm of receptor cooperativity between PAR2 and TLR4. We hypothesized that intracellular signaling pathways utilized

by TLRs and PAR2 would converge either cooperatively or non-cooperatively when co-engaged. We demonstrate herein the cooperation between PAR2 and TLR2, TLR3, or TLR4 for activation of NF-kappaB-dependent signaling in mucosal EC lines. In contrast, activation of PAR2 negatively regulated TLR3-dependent antiviral pathway, blunting the expression of TLR3/IRF-3-driven genes, as well as activation of IRF-3 and STAT1. Similarly, TLR4-TRIF-activated, IRF-3-driven genes were also attenuated by PAR2 co-activation. Consistent with these in vitro observations, PAR2 / and TLR4 / mice, which were refractory to footpad edema induced by PAR2 agonist peptide, were protected from mouse-adapted H1N1 influenza A virus-induced lethality when compared to wild-type mice; whereas, IFN-beta-/- mice were hypersusceptible. These data support and extend our recently described, novel model of PAR2-TLR4 receptor cooperativity and highlight the complexity of signaling integration between heterologous innate immune biosensors. Currently, experiments are being conducted to develop novel therapeutics for influenza infection in vivo using available inhibitors of TLR4 and a potent, patent-pending, small-molecule inducer of the IRF-3/IFN-beta pathway. (Ph.D work; Supported by NIH Grants T32-AI-07540 (QN), R37 AI-18797 (SV), R01 DK048373 (AF), R01 HL084387, R01 DK081376, R01 CA098369 (TA), R01 AI50632S1 (JT), and R01 AI050632 (DF))

19. HOLO-NI(II)HPNIKR CONTAINS ATYPICAL 5- AND 6-COORDINATE METAL BINDING SITES

Abby L. West, Sarah L.J. Michel

Poster Presentation; Room 349; Basic Science C

Abstract: The metalloregulatory protein NikR from Helicobacter pylori (HpNikR) is a master regulator of gene expression in Helicobacter pylori where it both activates and represses specific genes in response to nickel availability. The pleotropic gene regulation of this NikR homolog is unique to H. pylori where it facilitates efficient colonization of the human stomach. Here, we report a 2.3Å resolution structure of HpNikR resulting from crystals prepared from HpNikR as purified. This is the first crystal structure of HpNikR prepared directly from the holo-protein. The structure contains four nickel ions located at two distinct coordination sites. Two nickel ions are bound to a predicted four-coordinate square planar site while the remaining two nickel ions are bound at a novel site located that is ~ 5Å from the 4-coordinate site with a nickel-nickel distance of ~ 15Å. One of the nickel ions at this novel site is pentacoordinate (square pyramidal) and the other is hexacoordinate (octahedral). The novel site contains three histidines from two separate monomeric HpNikR units to coordinate nickel in addition to two water atoms for the pentacoordinate site and three water atoms for the octacoordinate site.

20. CHARACTERIZATION OF THE SEQUENCE FEATURES THAT INFLUENCE THE MTR4P-RNA INTERACTION

Jade Bernstein, Jeff D. Ballin, Dimeka N. Patterson, Gerald M. Wilson, Eric A. Toth

Poster Presentation; Room 349; Basic Science D

Mtr4p is a DEVH-box helicase required for 3'-end processing and degradation of various nuclear RNA substrates. In particular, Mtr4p is essential for the creation of 5.8S rRNA, U4 snRNA, and some snoRNAs, and for the degradation of CUTs, aberrant mRNAs and tRNAs. Many instances of 3'-end processing require limited polyadenylation to proceed. While polyadenylation can signal degradation in species from bacteria to humans, the mechanism whereby polyadenylated substrates are delivered to the degradation machinery is unknown. Our previous work has shown that Mtr4p has a preference for poly(A) RNA. We suspect that this preference aids in targeting polyadenylated

RNAs to the exosome. In these studies, we have investigated the mechanism underlying the preference of Mtr4p for poly(A) substrates as a means to understand how Mtr4p might facilitate targeting. Our analysis has revealed that Mtr4p interacts with poly(A) via a mechanism that is distinct from the mechanism used when it interacts with other substrates. In addition, we show that homopolymeric stretches like poly(A) suppress the ATPase activity of Mtr4p. Suppression of activity correlates with a decrease in the rate of complex dissociation. These findings indicate that the Mtr4p-poly(A) complex is unique and ideally suited for targeting to the exosome.

21. Cleavage of Protease-Activated Receptor-1 by Membrane Serine Proteases

KM Hodge, S. Netzel-Arnett, MS Buzza, TM Antalis

Poster Presentation; Room 349; Basic Science D

Protease-activated receptors (PARs) are a family of G-protein coupled receptors activated by proteolytic cleavage at the N-terminus, exposing a tethered ligand that initiates a conformational change of the receptor and activation of an associated G protein. There are four PAR family members of which PAR2 has been most extensively characterized for activation by different proteases. The activation of PAR1 has been implicated in disease mechanisms including invasion, extracellular matrix degradation, angiogenesis, and tissue remodeling in the cancer microenvironment. Thrombin, a blood coagulation protease, is the most studied activator of PAR1. Using an alkaline phosphatase reporter assay system to measure PAR1 cleavage, we show that Testisin activity on the cell surface leads to specific N-terminal cleavage of PAR1. We are generating and characterizing a recombinant soluble form of Testisin to compare with thrombin cleavage of PAR1. Our results suggest that membrane serine proteases may participate in the activation of PAR1 during angiogenic and malignant processes.

22. Effects of Commonly Used Excipients on the Expression of CYP3A4 in Colon and Liver Cells

Leslie Tompkins, Caitlin Lynch, Sam Haidar, James Polli, and Hongbing Wang

Poster Presentation; Room 349; Basic Science D

Purpose: The objective of this investigation was to assess whether common pharmaceutical excipients regulate the expression of drug-metabolizing enzymes in human colon and liver cells. Methods: Nineteen commonly used excipients were evaluated using a panel of experiments including cell-based human PXR activation assays, real-time RT-PCR assays for CYP3A4 mRNA expression, and immunoblot analysis of CYP3A4 protein expression in immortalized human liver cells (HepG2 and Fa2N4), human primary hepatocytes, and the intestinal LS174T cell models. Results: No excipient activated human PXR or practically induced CYP3A4. However, three excipients (polysorbate 80, pregelatinized starch, and hydroxypropyl methylcellulose) tended to decrease mRNA and protein expression across experimental models. Conclusion: This study represents the first investigation of the potential role of excipients in the expression of drug-metabolizing enzymes. Findings imply that some excipients may hold potential for excipient-drug interactions by repression of CYP3A4 expression.

23. ROLE OF A STRICTLY CONSERVED RESIDUE IN N-GLYCOSYLIC BOND CLEAVAGE BY HUMAN THYMINE DNA GLYCOSYLASE

Atanu Maiti and Alexander C. Drohat

Poster Presentation; Room 349; Basic Science D

Thymine DNA glycosylase (TDG) promotes genomic integrity by removing thymine from mutagenic G·T mispairs arising from deamination of 5-methylcytosine and follow-on base excision repair enzymes restore a G·C pair. Previous studies suggested that Asn140, a strictly conserved active site residue is important, for base excision, yet its catalytic role had not been investigated rigorously. To further our understanding of the catalytic mechanism of TDG, here we determined the contribution of Asn140 to the substrate binding and chemical steps of the reaction. Isothermal titration calorimetry (ITC) experiments show that TDG-N140A variant binds substrate analogs with the same tight affinity as wild-type TDG, indicating Asn140 does not contribute to substrate binding. Single turnover kinetics experiments show that TDG-N140A exhibits no detectable base excision activity for a G·T substrate, and its excision rate is vastly diminished (by ~104.4 fold) for G·U, G·FU, and G·BrU substrates. Our findings indicate that Asn140 is essential for the chemical step, but does not contribute substantially to substrate binding. Thus N140A variant provides a useful platform for investigating the role of other residues in forming the reactive enzyme-substrate complex.

24. CANAL PLANE CHARACTERIZATION AND ADAPTATION OF THE AVOR FOLLOWING TRAUMATIC BRAIN INJURY

M. Scherer, Schubert M

Poster Presentation; Room 349; Basic Science D

Recent findings in a U.S. Army Brigade Combat Team report that dizziness and imbalance both ranked among the top three symptoms for military Service Members (SM) who sustained traumatic brain injury (TBI) while deployed to Iraq. Despite the prevalence of vestibular- like complaints in this patient population, underlying pathology for these symptoms has not yet been established. We have initiated an independent line of research to characterize angular vestibulo-ocular reflex (aVOR) gain (eye velocity/head velocity) in blast-exposed SMs with TBI at Walter Reed Army Medical Center (WRAMC) using a wireless scleral search coil method (High velocity active and passive yaw and pitch plane head impulses) and a comprehensive battery of vestibular function tests. We also present data from an adaptation study conducted at The Johns Hopkins University Medical Center in which we characterized aVOR responses to rapid head impulses in each semicircular canal (horizontal, posterior, and superior) in a civilian patient (N= 1) with a moderate TBI after he completed vestibular rehabilitation including gaze and gait stabilization exercises to manage his complaints of vertigo and dizziness. RESULTS 1. Adaptation Study (Johns Hopkins University): Monocular wired scleral search coil recordings were used in the civilian patient subject to establish function in the semicircular canals to passive head impulses. The subject with TBI demonstrated elevated aVOR gains relative to an age-matched control ($p < 0.05$) in both posterior canals (1.05 vs. 0.97; 0.93 vs. 0.79) and to leftward impulses (horizontal canal, 0.90 vs. 0.82). Following passive assessment, each subject was exposed to a paradigm that progressively increased the aVOR gain with active head movements. aVOR adaptation was evident in all training trials for the patient subject ($p <0.05$, Tukey s HSD) and was retained for passive ($p < 0.05$) post adaptation testing. Adaptation was not retained for post adaptation active impulse testing ($p> 0.05$). In contrast, the control subject demonstrated significant aVOR gain adaptation during 7 of 10 training trials ($p<0.05$), which was preserved to both active and passive assessment ($p <$

0.05). 2. Characterization Study (WRAMC): Four blast exposed Service Members with TBI have been assessed to date using a wireless scleral search coil system to obtain aVOR gain to yaw and pitch plane impulses. Normal aVOR gain is typically 1.00 (+ .15). DISCUSSION and CONCLUSION Our results in a single subject with moderate TBI demonstrate abnormally high aVOR gains relative to an age-matched control. These findings are consistent with reports of elevated gains in other patient populations with CNS pathology affecting the VOR pathways (i.e. cerebellum lesions). Additionally, aVOR gain adaptation does not appear as robust in patients with TBI as that seen in subjects without head injury. Diminished retention of aVOR adaptation in patients with TBI could have significant implications for vestibular rehabilitation programs commonly prescribed to symptomatic SMs following blast exposure. aVOR gains in blast exposed SMs with TBI appear highly variable. Initial findings in this populaiton suggest that patient symptoms, head impulse test data, and the oculomotor exam during VFT are promising indicators of pathology. Translational research to characterize vestibular pathology remains a critical objective as we move toward evidence based diagnosis and management of blast exposed SMs with TBI and dizziness.

25. DUEL MECHANISMS FOR HYDROPHOBIC INTERACTIONS BETWEEN OBSCURIN AND SANK1

Chris D. Willis, B. Busby, T. Oashi, A.D. MacKerell, Jr, R. J. Bloch

Poster Presentation; Room 349; Basic Science D

Small ankyrin-1 (sAnk1, Ank1.5) is a splice variant of the ANK1 gene that binds to the large modular protein, obscurin A, with nanomolar affinity. Their binding may contribute to the architecture of the sarcoplasmic reticulum in striated muscle. A set of lysine and arginine residues in the two ankyrin repeats of sAnk1 interact specifically with four glutamate residues in a stretch of 30 amino acids of obscurin to mediate binding. Homology modeling and molecular dynamics simulations predict the presence of four hydrophobic residues in a single hot spot on the surface of the ankyrin repeat domain of sAnk1. We used site-directed mutagenesis of bacterially expressed fusion proteins, followed by blot overlays and surface plasmon resonance assays, to study the contribution of these four hydrophobic residues, V70, F71, I102 and I103, to binding to 30-mers of obscurin. Alanine mutations of each of these residues inhibited binding to residues 6316-6345 of obscurin (Obsc6316-6345). In contrast, V70A and I102A mutations had no effect on binding to a second site on obscurin, located within residues 6231-6260 (Obsc6231-6260). Using the same methods, we mutated the five hydrophobic residues present in Obsc6316-6345 to alanine and identified V6328, I6332, and V6334 to be critical for proper binding to wild type sAnk1. Our results suggest that hydrophobic interactions as well as electrostatic interactions are important for the binding of sAnk1 to Obsc6316-6345, consistent with studies of the complexes formed by other ankyrin repeat proteins with their ligands. On the contrary, alanine mutants of the six hydrophobic residues of Obsc6231-6260 did not alter binding to sAnk1. Therefore, hydrophobic interactions are likely to contribute to the difference in affinity of sAnk1 for Obsc6316-6345 and Obsc6231-6260, and to the dominant role played by the more C-terminal sequence in binding.

26. ABSENCE OF THE TYPE I IL4 RECEPTOR INCREASES THE SEVERITY OF AIRWAY INFLAMMATION IN A MURINE MODEL OF ASTHMA

Preeta Dasgupta, Elizabeth Smith and Achsah D. Keegan

Oral Presentation; Room 203; Basic Science E

The TH2 cytokines, IL-4 and IL-13, play critical roles in inducing allergic lung inflammation, eosinophilia, mucus hypersecretion and also drive alternative activation of macrophages (AAM). Although both cytokines share receptor subunits and signaling machinery, it is becoming apparent that IL-4 and IL-13 have differential roles in asthma pathogenesis. While clearly important in TH2 differentiation, the contribution of IL-4 signaling via the Type I receptor in allergic responses remains unclear. Here, we adoptively transferred wild-type ova primed CD4+ T cells into C57BL/6, Rag2 KO, or gamma c (³c) KO mice. ³c KO mice have functional Type II receptor complexes but lack the Type I receptor. We found that ³c KO mice developed increased inflammation around the airways and blood vessels in the lungs upon ova challenge when compared to WT and Rag2 KO mice. Moreover, significantly higher numbers of eosinophils were present in the bronchoalveolar lavage (BAL) and lung tissue in ³c KO mice. Immunohistochemical studies showed that all mouse strains expressed characteristic AAM proteins FIZZ1 and YM1 in lung epithelial cells, while macrophages expressed only YM1. Interestingly, the number of airways that were FIZZ1+ or YM1+ was significantly higher in ³c KO mice. These results suggest that the Type I R acts to suppress allergic lung inflammation and, in the presence of TH2 effectors, the Type II R can mediate allergic responses in its absence. Further experiments are required to fully elucidate the role of the Type I IL-4R and ³c in asthma. Supported by PHS grants AI038985 and AI059775.

27. BLIMP1 IS NOT REQUIRED FOR ALL IG SECRETION AND J-CHAIN EXPRESSION IN NURSE SHARK PLASMA CELLS

C. Doremus, H. Dooley, Y. Ohta, M. F. Flajnik

Oral Presentation; Room 203; Basic Science E

The small polypeptide, J-Chain or Joining Chain is involved in the multimerization of IgM and IgA in mammals. The oldest animals with an adaptive immune system complete with Ig isotypes, the cartilaginous fish, express IgM in two forms: the common pentameric (19S) form, which is attached to J-chain, and a monomeric (7S) form. By in situ hybridization adult nurse sharks have two populations splenic IgM-producing plasma cells; those that are J-chain positive, which are presumed to secrete pentameric IgM, or are J-chain negative and 7S secretors. Neonatal nurse sharks only produce pentameric IgM, and all plasma cells express J-chain. In addition, some neonatal shark plasma cells, found in discrete splenic locations, express a germline-joined isotype, IgM1gj. We hypothesize that during B cell development either 19S producers 'switch' to become 7S producers by shutting down J-chain expression or the two populations develop from separate lineages, with the 7S, J-chain-negative lineage arising later in development than the 19S and IgM1gj lineages. Identification of factors controlling J-chain expression, in shark B cells is necessary to clarify which B cell development pathway is correct. In mammals, Blimp-1 is required for development and maintenance of J-chain expression and immunoglobulin secretion in plasma cells. However, in situ hybridization data show that Blimp-1 is not expressed in neonatal IgM secreting cells, or in half of adult shark secretory cells, presumably the 19S producers. Our data imply that J-chain

expression and antibody secretion functions of some shark plasma cells are not controlled by BLIMP1.

28. TARGETING AUTOPHAGY ENHANCES THE ANTICANCER ACTIVITY OF THE RETINOIC ACID METABOLISM BLOCKING AGENT VN/12-1 AGAINST ENDOCRINE RESISTANT HUMAN BREAST CANCER CELL LINES

Abhijit M. Godbole, Vincent C.O. Njar

Oral Presentation; Room 203; Basic Science E

Breast cancer (BC) is the most common cancer and the second leading cause of cancer related deaths among women in the United States. Although endocrine therapies such as aromatase inhibitors have improved the overall survival, resistance to this therapy remains a major concern. Thus, there is a need to develop new and more efficacious therapies. VN/12-1, a novel retinoic acid metabolism blocking agent (RAMBA) developed in our laboratory, showed excellent anti-proliferative activity against endocrine sensitive MCF7 cell line. This led to the hypothesis that the compound may have activity against endocrine resistant breast cancer cells as well. To test this, the endocrine resistant breast cancer cell lines SK-BR-3 and MDA-MB-231 were treated with increasing concentrations of VN/12-1 and it was found that the compound inhibits the growth of both cell lines. However, it is ~10 fold less potent against endocrine resistant breast cancer cell lines compared to endocrine sensitive cell line MCF-7. Autophagy is a unique, regulated mechanism of catabolism of proteins under nutrient deprivation or chemotherapeutic stress. It is an important cause of resistance to chemotherapeutic agents. We wanted to investigate autophagy as a possible cause of reduced potency of VN/12-1 against endocrine resistant cell lines. The microarray technology previously employed to analyze gene expression changes in endocrine resistant LTLC cells treated with a structurally related RAMBA VN/14-1, revealed the upregulation of genes involved in endoplasmic reticulum stress (ERS) and intracellular membrane bound organelle formation (autophagosome). This led us to further hypothesize that VN/12-1 may also be causing similar changes in gene expression profiles of SK-BR-3 cells. Indeed, western blot analysis confirmed that VN/12-1 induced the expression and activation of genes involved in the ERS in SK-BR-3 cells. Activation of the ERS resulted in the upregulation LCB, an important marker of autophagy and other markers. Further characterization by electron microscopy and immunofluorescence revealed physical presence of autophagosomes. Pharmacological inhibition of autophagy by chloroquine enhanced the cytotoxicity of VN/12-1. The combination of VN/12-1 and chloroquine resulted in induction of intrinsic mechanism of apoptosis in SK-BR-3 cells. Pan caspase inhibitor z-vad-fmk blocked the cytotoxic effects of the combination of VN/12-1 and chloroquine. Significance: Autophagy is an important response of the cancer cells to ward-off the cellular stress caused by chemotherapeutic agents. Inhibiting autophagy blocks the protective response of the cells to stress and enhances the efficacy of the chemotherapeutic agent. VN/12-1 and its combination with autophagy inhibitor chloroquine is a novel therapeutic alternative to treat endocrine resistant breast cancer and hence warrants further pre-clinical and clinical development. *The RAMBA technology has been licensed to Cancer Research UK for further clinical development.

29. N-(2-HYDROXYLPROPYL) METHACRYLAMIDE (HPMA) COPOLYMER CONJUGATES FOR ACTIVE TARGETING TO HER2-OVEREXPRESSING TUMORS

Jun H. Lee

Oral Presentation; Room 203; Basic Science E

Current therapies of Human Epidermal Growth Factor Receptor type 2 (Her2) overexpressing cancers including breast cancer are limited by development of rapid drug resistance following treatment with monoclonal antibodies such as trastuzumab (TRZ), and the low selectivity and toxicity of small molecule tyrosine kinase inhibitors (TKIs) such as lapatinib. We hypothesize that covalent conjugation of these drugs to water-soluble N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer carriers with tailored side-chain and end-chain composition can potentially enhance the dose received by the tumor (increase efficacy) and minimize accumulation in non-target organs (reduce toxicity). Here we report the conjugation of HPMA copolymers with random or semitelechelic architecture to the anti-Her2 drug TRZ, subsequent physicochemical characterization and in vitro bioactivity evaluation. The HPMA-TRZ conjugates were successfully synthesized by radical polymerization techniques. The TRZ content and molecular weight distribution of the synthesized conjugates were consistent with their molar feed ratios. The in vitro antiproliferative efficacy of the conjugates were evaluated by administering as single agents or in combination with a model TKI (PKI166, Novartis) on a Her2 overexpressing BT-474 breast cancer cell. The polymer conjugates administered as single agents, inhibited cancer cell growth in a dose dependent manner and were comparable to the effect of free TRZ. Additionally, the combination treatments synergistically inhibited cancer cell growth when compared to treatment with single agents. Overall these studies demonstrate the potential of HPMA copolymer-TRZ conjugates in targeted drug delivery to Her2 positive tumors.

30. MULTIPLE ROLES FOR PROTEASE ACTIVATED RECEPTOR 2 (PAR2) ACTIVATION IN THE PROMOTION OF THE TH2 RESPONSE.

Jennifer A Stiltz; Notari, Luigi; Zhao, Aiping; Urban, Joseph F.; Sun, Rex; Shea-Donohue, Terez

Oral Presentation; Room 203; Basic Science E

Introduction: A number of enteric nematodes preferentially infect the upper small intestine (SI) and upregulate Th2 cytokines. The mechanisms involved in the initiation of Th2-mediated immunity remain unclear, but do not appear to require toll-like receptors (TLR). We showed previously that multiple nematode species produce a serine-like protease that increases mucosal permeability through activation of PAR2 and availability of PAR2 is critical for this effect. Enhanced permeability allows the passage of worm-derived products across the mucosal barrier and facilitates activation of antigen-presenting cells such as dendritic cells (DC). Aim: To determine the contribution of PAR2 to the development of host defense against nematode infection. Methods: To determine the distribution of PAR2 along the GI tract, expression was determined by real-time PCR in mucosal cells collected from the upper, middle, and lower SI and from the colon of uninfected BALB/c mice. To investigate the immune regulation of PAR2, nematode infection-induced changes in jejunal expression were measured at 9 days post Nippostrongylus brasiliensis (Nb) infection. PAR2 is required for DC maturation; therefore, we used JAWS II cells, an immortalized DC line, to assess the effects of PAR2 activating peptide (SLIGRL, 200nM) on PAR2 expression. Results were compared to lipopolysaccharide (LPS, 100ng/ml). Results: PAR2 expression was higher than TLR4 in the SI. Nematode infection induced a STAT6-dependent increase in the

expression of PAR2(1.0 ± 0.1 vs 2.7 ± 0.4 fold) associated with the increase in mucosal permeability. Nematode infection upregulated the expression of mucosal mast cell protease-1 (1.0 ± 0.3 vs 125 ± 50 fold), a known PAR2 agonist. Treatment of JAWS II cells (Table) with SLIGRL increased PAR2 expression. LPS alone had no effect on PAR2 expression, but significantly amplified the effects of SLIGRL. Conclusions: These data suggest that 1) preferential expression of PAR2 vs TLR4 in the SI favors nematode interactions with PAR2; 2) the STAT6-dependent increase in PAR2 tissue expression during Nb infection serves to maintain PAR2 availability through a positive feedback mechanism; and 3) infection increases the availability of PAR2 agonists that increase permeability and promote DC maturation by upregulating PAR2 expression on DCs. This effect can be amplified by LPS suggesting a novel interaction between TLR4 and PAR2 in DCs.

31. CONTROL OF SEQUENTIAL ACTIONS IN TYPICALLY DEVELOPING CHILDREN: PREDICTIVE CONTROL AND PERFORMANCE ASYMMETRY

P. Viswanathan and J. Whitall

Oral Presentation; Room 203; Basic Science E

The present study investigates the age-related changes in motor control and performance asymmetry in typically developing (TD) children, concerning control of sequential actions when reaching to point, grasp, lift and place an object. We hypothesized that the feedforward and feedback components embedded in the reach would show the effect of increasing movement components only in the older age groups and that younger children would be symmetric. We tested a developmental landscape of children in the age range of 5-11 years (n=24) and 8 young adults. We used a motor screen (Movement Assessment Battery for Children) and used a cutoff of greater than 20% to assess that the motor development was age-appropriate. All participants performed the following four conditions, with each arm, in a randomized order: (i) an isolated reach to point, (ii) a reach-to-grasp, (iii) reach-to-grasp and lift and (iv) reach-to-grasp, lift and place tasks in a modified discrete Fitts Law paradigm. Participants were asked to move as fast and accurately as they could. We also calculated separate indices of difficulty for each subject to enable ease of comparison. Scaling the experiment tasks to body proportion yielded similar indices of difficulty values across all the age groups (4.7-5.4). As compared to adults and older children, 6-year-old children showed reduced feedforward control associated with slower, jerkier movement profiles, longer transition times and lack of anticipatory modifications in control of integrated task sequences. The 6-year-old children showed a preferred hand advantage only for movement time and spatial accuracy of hand trajectory path. Contrary to expectations, the older children were not completely adult-like in the feedforward control and asymmetry profiles, suggesting a differential developmental trajectory for feedforward and feedback control mechanisms in control of integrated task sequences.

32. ISOFORM-SPECIFIC RNA-BINDING PROPERTIES OF THE MRNA-DESTABILIZING FACTOR AUF1

Beth E. Zucconi, Jeff D. Ballin, Brady Y. Brewer, and Gerald M. Wilson

Oral Presentation; Room 203; Basic Science E

AUF1 binding to AU-rich elements (AREs) in the 3'-untranslated regions of mRNAs encoding many inflammatory cytokines and other regulatory proteins leads to changes in mRNA stability, which influences protein expression levels. Many proteins regulated through this post-transcriptional control

mechanism are involved in cardiovascular disease and tumorigenesis. AUF1-mRNA association is a dynamic paradigm directed by various cellular signals, but many features of its function remain poorly described. AUF1 is expressed as a family of four protein isoforms resulting from alternative splicing of exons 2 and 7 from AUF1 pre-mRNA. Recent literature reports some preliminary evidence that different isoforms have varied functional characteristics. We hypothesized that isoform-specific variations in AUF1 function may be the result of biochemical differences in their RNA-binding activities. To test this model, we generated highly purified recombinant forms of each AUF1 isoform and validated their dimerization status by chemical crosslinking and gel filtration. Subsequently, we determined the binding affinity and association mechanism of each isoform for ARE-containing RNA substrates by fluorescence anisotropy and electrophoretic mobility shift assays, and protein-dependent RNA structural remodeling activities by fluorescence resonance energy transfer (FRET). Our data reveal that protein sequences encoded by each of the two alternatively spliced AUF1 exons confer distinct consequences on AUF1 interactions with RNA. First, retention of exon 2-encoded sequences inhibits RNA-binding activity. By contrast, inclusion of exon 7-encoded sequences enhances RNA-dependent formation of protein oligomers. Finally, saturated RNA:protein complexes involving exon 7-retaining AUF1 isoforms maintain associated RNA substrates in a relatively open conformation, while AUF1 isoforms lacking exon 7-encoded sequences promote condensed local RNA structures. These data are pivotal since changes in local RNA structure can occlude or expose binding sites for other ARE-binding proteins or microRNAs that may have cooperative, dominant, or antagonistic functions to that of AUF1. Furthermore, these findings may present opportunities to regulate AUF1 function by isoform-specific manipulation of its RNA-binding or RNA-remodeling activities.

33. STUDY OF ATP13A2 PROTEIN INVOLVED IN PARKINSON'S DISEASE

Janet Ugolino and Mervyn Monteiro

Oral Presentation; Room 223; Basic Science F

Study of ATP13A2 protein involved in Parkinson's disease Janet Ugolino and Mervyn J. Monteiro Mutations in ATP13A2 have been linked to Kufor-Rakeb syndrome, a form of juvenile parkinsonism with dementia. The ATP13A2 gene is highly expressed in the brain and encodes a protein with high homology to P-type ATPases, which are thought to function as ion pumps. A previous study indicated that wild type ATP13A2 protein localized to lysosomes whereas three different ATP13A2 mutant proteins appeared to be retained in the ER. One speculation is that the mutant ATP13A2 proteins are misfolded and are retained by the quality control machinery of the ER. Misfolded proteins retained by the ER are usually retro-translocated to the cytoplasm for rapid degradation by the ubiquitin-proteasome pathway in a process known as ER-associated degradation (ERAD). In mammalian cells, ERAD is a complicated process and it is not well known which ERAD protein complexes are responsible for facilitating the degradation of specific ERAD substrates. We questioned whether ATP13A2 mutant proteins were degraded by ERAD and which ERAD proteins were involved in this process. We hypothesized that knockdown or inhibition of important ERAD proteins would stabilize the degradation ATP13A2 proteins. To test this hypothesis, we measured the degradation rates of transfected wild type and two mutant ATP13A2 proteins in HeLa cells following siRNA knockdown of erasin, an ERAD protein characterized in our lab. Knockdown of erasin slowed the turnover of the mutant proteins, although the amount of

stabilization differed for each mutant. This suggests ATP13A2 mutants are degraded by different ERAD complexes. Expression of a dominant negative mutant form of p97/VCP, a protein that is essential for ERAD, also stabilized the degradation of ATP13A2 mutants. We also questioned the ubiquitination state of the ATP13A2 proteins. The previous study showed stabilization of the proteins with proteasome inhibition but failed to detect ubiquitination of the proteins which is a hallmark of the cell's protein degradation pathways. To address this issue, ATP13A2 proteins were immunoprecipitated from transfected HeLa cells and ubiquitination of the proteins was detected via Western blot. Ubiquitinated ATP13A2 proteins were detected after inhibition of the proteasome with MG132. Ubiquitinated ATP13A2 proteins were also detected upon co-expression of the dominant negative p97/VCP mutant although the amount of ubiquitination differed between mutants. The above results indicated that ATP13A2 proteins may be degraded by different mechanisms. Further study of these proteins will elucidate the mechanism of ATP13A2 degradation and provide further insight into mammalian ERAD.

34. SEROTONERGIC MODULATION OF GLUTAMATERGIC TRANSMISSION IN DEPRESSION

Angy Kallarackal, Xiang Cai, Scott Thompson

Oral Presentation; Room 223; Basic Science F

Depression is a widespread psychiatric disorder affecting 10% of the world population, but the causes of depression and the mechanisms underlying cognitive dysfunction remain unknown. The most common treatments for depression, selective serotonin reuptake inhibitors (SSRIs) are only truly effective in 50% of patients. Although SSRIs increase serotonin levels rapidly, therapeutic effects take up to 3-4 weeks, raising the question as to what downstream factors mediate their efficacy. One potential mechanism is a change in the ability of 5-HT1BR serotonin receptors to potentiate AMPAR-mediated synaptic responses at temporo-ammonic synapses with CA1 cells in the hippocampus, as we have recently discovered. In the current study, we tested 5-HT1BR-induced potentiation in the chronic unpredictable stress (CUS) model of depression. Activation of 5-HT1BRs with the agonist anpirtoline strongly, but reversibly increased temporo-ammonic (TA)-CA1 EPSPs in acutely prepared hippocampal slices from control animals. This effect was enhanced 2-fold in slices from CUS animals and was unable to be reversed upon washout. If alterations in 5-HT1BR-mediated potentiation contribute to the pathology of depression, then opposite changes should be produced by chronic antidepressant treatment. Indeed, fEPSPs in hippocampal slices from animals treated with fluoxetine or imipramine for 21-28 days were unable to be potentiated by anpirtoline. These results reveal a novel and striking change in synaptic transmission in an animal model of depression. Investigation of these processes provides insight into how stress-induced changes may produce the behavioral phenotype of depression and how they may be ameliorated by antidepressant treatment.

35. SYNAPTIC AMPA RECEPTOR DISTRIBUTION IS CONTINUOUSLY DYNAMIC AND COORDINATED WITH POSTSYNAPTIC DENSITY SCAFFOLD PROTEINS

Justin M Kerr, Thomas A Blanpied

Oral Presentation; Room 223; Basic Science F

Efficient communication at synapses requires precise alignment of the presynaptic neurotransmitter release machinery with postsynaptic neurotransmitter receptors. Receptors are positioned in the postsynaptic density

(PSD), a dense protein matrix thought to anchor receptors in place and tie them to synaptic signaling molecules. The core of the PSD is organized by scaffold proteins that regulate AMPA receptor number, synaptic strength, structural plasticity, and recruitment of intracellular signaling molecules. Previous work has shown that individual PSDs act as a flexible matrix, and undergo continuous morphological change on a time scale of seconds to minutes. This strong, actin-dependent deformation of the PSD suggests that the subsynaptic distribution of AMPA receptors may not be stable. However, it is not known whether acute alterations of the postsynaptic matrix directly regulate synaptic receptor positioning. To test this, we utilized live-cell confocal imaging of mature, cultured hippocampal neurons expressing fluorescently tagged AMPA receptors and PSD scaffold proteins. At single synapses, the AMPA receptor cluster underwent strong and continuous changes in morphology, and these changes were coordinated with concurrent variation in morphology of the PSD. Furthermore, the distribution of AMPA receptors within individual synapses varied over time. We hypothesized that, like PSD morphological dynamics, ongoing changes in synaptic AMPA receptor distribution require actin polymerization. Consistent with this notion, pharmacological stabilization of actin filaments with jasplakinolide rapidly halted both PSD and AMPA receptor morphological changes with no change in pHluorin-GluR1 fluorescence intensity. Moreover, destabilization of F-actin with latrunculin-A rapidly decreased both PSD and AMPA receptor morphological dynamics, but initiated only a slow loss of synaptic receptor fluorescence. Thus, actin-driven changes in synapse morphology represent a novel mechanism for regulating short-term synaptic receptor distribution, distinct from the long-term maintenance of total synaptic receptor number. These experiments extend beyond traditional measures of spine morphology by directly imaging the morphology of synapse structure itself. Continual change in the distribution of synaptic AMPA receptors is likely to alter their spatial relationship to presynaptic glutamate release sites and access to intracellular signaling complexes, kinases, and phosphatases. As a result, fine scale morphological changes are likely to play an important role in regulating synaptic transmission and synaptic plasticity.

36. CANDIDA ALBICANS AND STAPHYLOCOCCUS AUREUS MIXED SPECIES BIOFILMS MEDIATE INVASIVE STAPHYLOCOCCAL INFECTION

BM Peters,MA Jabra-Rizk, ME Shirtliff

Oral Presentation; Room 223; Basic Science F

In nature, microbes rarely grow as mono-species planktonic forms. Rather, most are associated as polymicrobial biofilms attached to host and environmental surfaces. The polymorphic fungus Candida albicans and bacterium Staphylococcus aureus are both capable of forming biofilms, can be co-isolated from virtually all human mucosal sites, and are responsible for diverse localized and deep-seated infections. Despite causing a large number of mono-species infections, they have been implicated as co-infecting organisms in a variety of human diseases. Therefore, various approaches were used to understand the intricacies of this medically relevant dual-species biofilm. Confocal scanning laser microscopy and species specific PNA-FISH analysis revealed that S. aureus possesses an affinity for the pathogenic hyphal form of C. albicans in vitro. C. albicans hyphae are directly invasive and have been demonstrated previously to penetrate host epithelial tissue; therefore, we hypothesized that S. aureus may use the hyphae of C. albicans to become invasive. In vitro co-infection of human keratinized epithelial cells demonstrated the ability of S. aureus to attach to C. albicans hyphae and penetrate epithelial cells, while cells infected with S.

aureus alone showed no such breach of the epithelial surface. A similar invasive pattern was also demonstrated during infection of explanted murine tongue tissue. An in vivo mouse model of oral co-infection established the ability of S. aureus to translocate to the kidneys only in the presence of C. albicans while mono-species infections were either cleared or remained localized. Furthermore, through the use of various C. albicans cell wall protein knockout mutants, we have demonstrated that staphylococcal-hyphal binding is partially dependent on the candidal adhesin Als3p. Therefore, we present a novel mechanism of invasive S. aureus infection facilitated via interactions with C. albicans in an oral polymicrobial biofilm setting. These findings have significant impact on the consideration of treatment options for those afflicted with complicated biofilm mediated infections.

37. CONTROL OF FRANCISELLA LIPID A MUTANT INFECTION IS MEDIATED BY INTERFERON-GAMMA BUT NOT MYD88 SIGNALING OR ADAPTIVE IMMUNITY

Daniel A. Powell, Duangjit Kanistanon, Ilana E. Cohen, Adeline M. Hajjar, Mark Pelletier, Shawn J. Skerett, and Robert K. Ernst

Oral Presentation; Room 223; Basic Science F

Francisella tularensis subspecies tularensis (Ft) is an intracellular Gram-negative bacterium and the causative agent of the disease tularemia in humans. Ft is classified as a Category A select agent for its potential use in bioterrorism. F. tularensis subsp. novicida (Fn) is virulent in mice and is widely used to study pathogenesis and immune response to Francisella infection. Francisella lipid A, a biologically active component of lipopolysaccharide (LPS), has no to low endotoxic activity. A Fn lipid A biosynthesis mutant (lpxF-null mutant) lacks the 4'-phosphatase enzyme and its lipid A retains a phosphate moiety at the 4c position. This mutant was previously shown to be highly avirulent in the footpad infection model. The aims of this study were to determine the virulence and protective efficacy of lpxF-null mutant Fn in mice using subcutaneous and pulmonary routes of infection, and to elucidate the component(s) of the immune system involved in control of lpxF-null mutant infection. C57BL/6 mice infected with this lpxF-null mutant subcutaneously or via the pulmonary route survived the infection and developed protective immunity against lethal wild type Fn challenge. Subcutaneous heterologous challenge of immunized mice with type A and type B Ft showed no cross-protection. C57BL/6 and RAG-1-/- mice were subcutaneously inoculated with ~ 106 cfu of lpxF-null mutant Fn and all mice survived the infection, indicating that T and B cells are not required for the control of lpxF-null mutant infection. Components of the innate immune system were also examined, including TLR2, TLR4, caspase-1, MyD88, interferon-alpha (IFN-±) and interferon-gamma (IFN-³) using knockout mice. Only IFN-³ knockout mice succumbed to lpxF-null mutant infection, whereas MyD88 signaling was not essential in the control of this infection. In vivo neutrophil depletion using anti-Gr-1 antibody did not affect the outcome of lpxF-null mutant infection. In conclusion, IFN-³ production is important in control of Fn lipid A mutant infection. Sensing of this Fn lipid A mutant was independent of the TLR2, TLR4 and MyD88 pathway. The source(s) of IFN-³ in this infection remain to be determined.

38. EXPLORING THE FACTORS THAT INFLUENCE FUNCTIONAL PERFORMANCE AMONG NURSING HOME RESIDENTS

Valerie Sabol

Oral Presentation; Room 353; Informatics/Policy/Social Science A

Background: Prior to the Omnibus Budget Reconciliation Act (OBRA) of 1987, which mandated that nursing home (NH) residents attain and maintain their highest level of

function, functional impairment and disablement were generally accepted as normal consequences of aging, with minimal resources directed towards restorative care interventions. To promote healthy aging and to comply with OBRA, it is important to identify factors that impact functional performance, such as chair rise, in older NH residents. Objectives: Using the Disablement Process Model, factors influencing chair rise physical function were examined. It was hypothesized that variables from all levels of the Disablement Process Model would significantly impact the ability of a NH resident to get up from a chair. Method: A secondary data analysis, using stepwise multiple logistic regression, tested the impact of socio-demographic factors, active pathology factors such as anemia, functional and impairment factors, and psychosocial and environmental intervention factors on chair rise functional performance among 492 NH residents (from 12 different NHs in the state of Maryland) aged 65-102 years. Results: Analysis indicated that three factors, representing functional impairment (strength), functional limitation (gait), and psychosocial (intra-individual) intervention factors (self-efficacy) were identified as significant predictors in classifying chair rise and together explained approximately 64% of the variance for this functional task. Discussion: These findings indicate that identifying certain physical and psychosocial variables early in the disablement process will help healthcare providers tailor both medical and restorative care interventions that may limit or reverse functional impairment and/or disablement, and allow for maximal functional independence.

39. THE TRANSMEMBRANE DOMAIN I OF THE HUMAN APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER (HASBT; SLC10A2) CONTRIBUTES TO SUBSTRATE TRANSLOCATION EVENTS AND PROTEIN STABILITY

Tatiana Claro da Silva, Naissan Hussainzada, Chandra M. Khantwal, and Peter W. Swaan

Oral Presentation; Room 223; Basic Science F

The human apical sodium-dependent bile acid transporter (hASBT, SLC10A2) is the chief bile acid transporter in the intestines, from where it reclaims bile acids via sodium co-transport, thus playing a critical role in the enterohepatic circulation of bile acids, as well as in cholesterol homeostasis. Disease states, such as gallstone formation, and PBAM, are associated with defects on ASBT function. In the present study, we employed a combination of mutagenesis, thiol modification (SCAM), kinetics and proteomics approaches, in an effort to understand ASBT structure-function requirements, focusing on its highly conserved, relatively amphipatic transmembrane domain 1 (TM1; Asn29-Gly50). Additionally, we included N27 and N28 in our investigations, due to their close proximity to TM1 aqueous-membrane boundary. Site-directed Cys replacement helped us establish the importance of this TM on ASBT function, with about 60% of the protein affected by mutation. Likewise, N27C impaired function suggested a critical role for N27 on protein function, whereas N28 remained unaffected. When probed with MTSET, residues clustered along the TM1 exofacial half clearly showed solvent accessibility, and involvement of L34 in bile acid interactions, and L38 and M46 in sodium transport was corroborated by further bile acid and cation protection experiments, as well as kinetics analysis. In addition to the structure-function findings, inhibition of proteasomal degradation, and the use of the chemical chaperone DMSO,

strongly suggest G50 role on the dynamic protein folding. Overall, our observations indicate a pivotal role for TM1 on ASBT function and stability. Supported by NIH RO1 DK61425.

40. EVIDENCE-BASED PRACTICE: ATTITUDES AND KNOWLEDGE OF SOCIAL WORKERS ACROSS GEOGRAPHIC REGIONS

Tina Abrefa-Gyan

Oral Presentation; Room 353; Informatics/Policy/Social Science A

Objective: To examine possible differences in knowledge and attitudes toward evidence-based practice (EBP) among social workers across geographic regions. Method: A random national sample of 180 NASW members was obtained from mail and Internet groups. MANCOVA analysis was performed to determine possible differences in knowledge and attitudes toward EBP among these social workers. Results: Controlling for age, findings suggest that knowledge and attitude towards EBP did not differ among these practitioners. Conclusion: Despite increasing efficacy and widespread knowledge of EBPs, there is little or no empirical evidence to support any differences in attitudes and knowledge of EBP among social workers across geographic regions. Keywords: Evidence-based practice, knowledge of EBP, attitude towards EBP, EBP knowledge across geographical regions. Overview: The wave of interest in evidence-based practices (EBP) has swept across the social services and gained widespread acceptance among researchers and policy makers alike (Fixen, Naoom, Blase, Friedman, & Wallace 2005). However, using EBPs has not been an easy task for professionals in any discipline (Mullen, Shlonsky, Bledsoe, & Bellamy, 2005). Social workers in particular are less likely to practice evidence-based interventions as compared to allied professionals (Gambrill, 2001; Mullen et al., 2005; Proctor & Rosen, 2004). Therefore, there is a gap between the social work research and practice communities, which widens when assessing the implementation of research findings related to evidence-based practice (Hagell & Spencer, 2004). Although social workers have identified the barriers and supports for embracing the use of evidence-based practice within their work settings (Gioia & Dziadosz, 2008), it is unknown how differ across geographic regions affect EBP adoption, even though some studies have examined how EBP affected the professional status of rural social workers in multidisciplinary health teams in Victoria, Australia (Murphy & McDonald, 2004). In this study, data from a study of National Association of Social Workers (NASW) members were analyzed to ascertain whether there are possible differences in knowledge and attitudes toward EBP among social workers in geographic areas.

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41. Assessing the Role of Attitudes, Knowledge, and Organizational Climate in the Use of Evidence-Based Practice among NASW Members

Saltanat Dushalieva

Oral Presentation; Room 353;
Informatics/Policy/Social Science A

The social services field has moved toward widespread endorsement of evidence-based practices (EBP) as part of a larger movement to better integrate clinical research findings into applied settings and plan programs based on empirical data, to improve standards of evaluating their practice and enhance accountability, and to ensure better outcomes for clients. Despite the various calls for the use of EBPs in clinical practice, these approaches appear to be underutilized in applied settings. Using the sample of 169 members of National Association of Social Workers (NASW), this paper assesses the role of attitudes, knowledge, and organizational climate in the use of EBP among NASW members. The study also examines the moderating effect of organizational climate on the relationship between attitudes towards and use of EBP. Results indicate that implementation of EBP in an organization is clearly influenced by practitioners attitudes, knowledge, and perceptions of their organizational environment in relation to the use of scientific evidence. The study also supports the validity of the 5Cs theoretical framework. All aspects of EBP implementation, incl. practitioners individual characteristics, competencies, and organizational context need to be utilized in order to create the structure favorable for development and management of EBP as a new approach.

42. The Cost-Effectiveness of Genotyping CYP 2C19 to Guide Antiplatelet Therapy Selection

Emily S. Reese, C. Daniel Mullins, Amber L. Beitelshees, Ebere Onukwugha

Oral Presentation; Room 353;
Informatics/Policy/Social Science A

BACKGROUND: The recent re-label of clopidogrel to include information on CYP2C19 genotype and the approval of a second-generation antiplatelet medication, prasugrel, could greatly impact the way antiplatelet therapy is prescribed. This study assesses the cost-effectiveness of genotyping patients to guide selection of antiplatelet therapy with clopidogrel or prasugrel. METHODS: A decision tree was created using prevalence of CYP2C19 metabolism status, cardiovascular events, bleeding events, and costs of events as reported in the literature and publically available FDA advisory committee documents. In the genotype arm, an individual s metabolic status determined medication selection. Effect was defined as event avoided. Scenario analyses conducted to determine the robustness of the model included scenario A: clopidogrel use without genotyping, scenario B: prasugrel use without genotyping, and scenario C: generic clopidogrel. Number needed to genotype to avoid one cardiovascular or bleeding event from occurring was also determined. RESULTS: The probability of being an ultra-rapid or extensive metabolizer of CYP2C19 was 73% and the probability of being an intermediate or poor metabolizer was 27%. For the no-genotype arm, the estimated proportions of medication selection used were 70% to receive clopidogrel and 30% to receive prasugrel. The model favored genotyping to determine antiplatelet therapy (ICER: $7,762). Scenario analyses exhibited a dominant strategy of genotyping patients (ICERA: $1,579, ICERB: $26,942, and ICERC: $6,735). Number needed to genotype to avoid one bleeding or

cardiovascular event was 26. CONCLUSION: The cost-effectiveness analysis suggests it is more effective but not less costly to genotype patients prior to selecting clopidogrel or prasugrel for the patient s antiplatelet therapy.

43. Insulins and Risk of Cancer among Type 2 Diabetics

Zhiqiang Lu, BPharm; Sheila Weiss Smith, PhD, FISP

Oral Presentation; Room 353; Informatics/Policy/Social Science A

OBJECTIVES: There have been reports which suggest that insulin glargine use may contribute to increased cancer risk in some type 2 diabetic populations, in addition to the fact that diabetes mellitus is also associated with certain types of cancer. Therefore, the objective of the present study is to conduct a review of published studies on insulins and the risk of cancer in patients with type 2 diabetes and summarize the findings. METHODS: Several databases such as Medline and PubMed were used for publication searching. Key words included insulin, tumor, cancer, type 2 diabetes, and specific insulin names such as glargine, lantus, lispro, aspart, etc. Only English language literature was considered for articles looking at the increased risk of cancer among type 2 diabetic patients using insulins. RESULTS: About 30 articles were selected, among those, 4 studies were conducted in humans using secondary database. All were historic cohort studies, and three used Cox regression for analysis. Two articles established a positive association between cancer incidence and insulin glargine, while the other two found no association. Three studies also showed that several insulins other than glargine are not associated with an increased risk of cancer. Limitations of these studies include failure to control for important confounders, small sample size and short study period, which may have impact on the risk of cancer. CONCLUSIONS: Given the fact that cancers are rare and often take a long time to develop, further studies require very large population with long follow up time to have sufficient power to detect a possible effect. This, combined with small proportion of insulin users who were exposed to glargine, may be a reason to studies that found no association, which leaves a question of a class effect. Future studies to explore the effect of all other insulins and the possible mechanism may help to untangle this question.

44. Challenges Performing Literature Database Searches Using PubMed

Elicia Preslan, Ashish Joshi

Oral Presentation; Room 353; Informatics/Policy/Social Science A

A simple approach was used to search PubMed literature (January 2000 to December 2009) focusing on bladder cancer risk factors, including environmental exposures, health status, lifestyle behaviors or sociodemographic factors. Key words were selected from four sources to evaluate the accuracy and precision of terms from each source, and searches were run using "urinary bladder cancer" plus a risk factor term. All search results were manually screened to identify quality articles. Risk factor keywords from MeSH entry terms resulted in an average of 24% accuracy in locating quality articles, while words chosen by the searcher had a 15% accuracy. Terms found lower on the MeSH trees and synonyms from a thesaurus had an average of 8% and 9% accuracy, respectively. These results demonstrate that for PubMed users conducting searches on a general topic or using free text search strings, a larger number of searches must be conducted in order to locate the desired articles.

45. Impact of Dementia on Medication Use and Adherence among Medicare Beneficiaries with Congestive Heart Failure

Sarah Dutcher, BS; Ilene Zuckerman, PharmD, PhD; Gail Rattinger, PharmD, PhD; Linda Simoni-Wastila, BSPharm, PhD; Stephen Gottlieb, MD; Bruce Stuart, PhD

Poster Presentation; Room 349; Basic Science C

Research Objective: This study describes medication use and adherence among older Medicare beneficiaries with CHF and evaluates the impact of a comorbid dementia diagnosis on medication adherence patterns in this population.
Methods: Using the MarketScan Medicare Coordination of Benefits data files, we conducted a retrospective analysis of Medicare beneficiaries diagnosed with CHF. Beneficiaries were ≥65 years, had a CHF diagnosis in 2006, and were continuously enrolled from 1/1/06-1/31/07. Adherence was assessed for evidence-based drugs indicated for chronic use in the treatment of systolic CHF. Our two year adherence measures included: 1) binary measure of use (prevalence); 2) continuous measure of days of therapy (duration); and 3) medication possession ratio (MPR). We describe the cohort and medication adherence patterns, and apply multivariable regression to assess impact of dementia on any use and duration of CHF treatment, adjusted for sociodemographics, comorbidities, healthcare utilization, and loss to follow-up.
Results: Of the 117,510 beneficiaries with CHF, the mean age was 78.5 (±7.3) years, 51.4% were female, and 80.4% filled >1 prescription for a chronic use medication. Among those receiving medication, mean MPR was 0.89 (±0.14). The 7.5% of individuals with a diagnosis of dementia were older (82.6 vs. 78.1 years), disproportionately female (58.6% vs. 50.9%), and more often had 'unspecified' CHF diagnoses (92.6% vs. 88.3%), compared to patients without dementia. Chronic CHF medication prevalence was lower in patients with dementia compared to patients without dementia (67.7% vs. 81.4%). Duration was significantly shorter for those with both diagnoses (460 vs. 565 days), though MPR did not differ. In regression analyses, dementia was associated with 14% lower prevalence and 11% shorter duration of chronic CHF medication use (both $p<0.0001$).
Conclusion/Implications: Although Medicare beneficiaries with CHF exhibit good medication adherence, findings suggest that dementia status may negatively affect treatment decisions for chronic diseases such as CHF. Further investigation into reasons for disparate treatment patterns and their impact on outcomes is warranted.

46. Iron Coordination to ZIF268: A Novel DNA Cleaver?

Jamie Michalek, Seung Jae Lee, Steven Rokita, Sarah Michel

Poster Presentation; Room 349; Basic Science A

Zinc fingers of Cys2His2 class are one of the most common and important DNA-binding motifs found in eukaryotes. ZIF-268, a classical zinc finger protein, uses a Cys2His2 ligand set to bind zinc and fold. ZIF-268 and DNA binding properties are well understood and it is possible to modify the protein sequence to tune DNA recognition.
We hypothesize that iron substituted ZIF-268 may be capable of cleaving target DNA possibly with sequence selectivity. Work to address this hypothesis, we have been over expressed and purified ZIF-268 and characterized its iron (ferric and ferrous) and zinc binding properties. We have learned that ZIF-268 binds DNA with the same affinity when zinc, ferric and ferrous iron is bound to its binding partner DNA (5'-GCGCGGGCG). In addition, ferrous coordinated TTP my be detrimental to the cell because it is redox active and may

generate damaging reactive oxygen species (ROS). We measured oxidation rates of zinc finger proteins as a function of metal ion coordination and have discovered that iron substitution promotes oxidation via the formation of reactive hydroxyl radicals. DNA footprinting assays proved that hydroxyl radical attacks the deoxyribose sugars arrayed along the surface of the DNA. This information could be further utilized for the search of target specific gene cleavage.

47. The role of CAR in the induction of DME by oxazaphosphorines

Duan Wang and Hongbing Wang

Poster Presentation; Room 349; Basic Science D

Cyclophosphamide (CPA) and ifosfamide (IFO) are oxazaphospharines-class prodrugs. CYP2B6 and CYP3A4 have been demonstrated to be the major CYP450s responsible for their biotransformation in the liver. Autoinduction effect has been observed in CPA and IFO, through which they can up-regulated the mRNA and protein levels of CYP2B6 and CYP3A4, thus promoting their own metabolism. Our research shows that the activation of human constitutive androstane receptor (CAR) played different roles in the autoinduction effect of CPA and IFO.

48. An Analysis of the Effect of Waiting Period on Substance Abuse Treatment Completion in Pregnant Women

Brianna Lindsay, Jennifer Albrect, Mishka Terplan

Oral Presentation; Room 353; Informatics/Policy/Social Science A

Very little research has been done evaluating factors that affect substance abuse treatment completion, particularly in pregnant women. A retrospective cohort study was conducted using the Treatment Episode Data Set-Discharges, 2006 (TEDS-D) database to analyze the association between days waiting to enter substance abuse treatment and program completion in pregnant women. The opportunity to improve treatment retention rates and in turn, health status of the mother and infant make this research especially warranted. The TEDS-D data set is publicly available from the United States Department of Health and Human Services, Substance Abuse and Mental Health Services Administration and provides treatment information routinely collected by state-funded agencies concerning individuals' drug treatment admissions and discharges. We restricted the data set to pregnant women. Additionally, information concerning how many days had elapsed from the first contact or request for service until the client was admitted to treatment was recorded. Our primary outcome of interest was substance abuse treatment completion or transfer to another facility. Because pregnancy represents a unique opportunity for substance abuse treatment intervention, we hypothesized that a shorter waiting period for treatment is associated with increased completion in treatment programs. Statistical analysis was performed to identify potential interactions and confounding covariates and to describe associations using odds ratios. Covariates studied included age, race, prior treatment, and referral status. Adjusted odds ratios were calculated using Mantel-Haenszel analysis and logistic regression. Further research should be conducted in order to conclude whether there is an association between time to enter treatment and completion.

49. Elucidating the APE1 & BCL2 Interaction

Brittney A. Manvilla & Alex C. Drohat

Poster Presentation; Room 349; Basic Science B

Apurinic/apyrimidinic endonuclease 1 (APE1 or Ref-1) is a multi-functional protein involved in several important cellular pathways. The most predominant function of APE1, its AP endonuclease activity, is essential for DNA base excision repair. APE1 hydrolytically cleaves the phosphodiester backbone 5' to AP sites arising spontaneously or due to the excision of damaged bases by a DNA glycosylase producing a 5'-deoxyribose phosphate and a 3'-OH primer for repair synthesis. Subsequent base excision repair enzymes complete the repair process through incorporation of the correct nucleotide. APE1 is essential for proper embryonic development and cell survival, and its overexpression confers resistance to pro-apoptotic stimuli including DNA damaging agents. DNA damage (and the ensuant appearance of AP sites) can be incurred through a variety of mechanisms including alkylation, UV irradiation, or exposure to oxidizing reagents such as NNK, a lethal carcinogen found in cigarette smoke. In recent years, BCL2, a pro-survival oncogene, was found to play an inhibitory role in the repair of NNK-induced AP sites through direct interaction with APE1. We are currently investigating the interaction between APE1 and BCL2 (BCL2/BCL-XL chimera) using NMR, fluorescence spectroscopy, native PAGE, size exclusion chromatography, and kinetics techniques. Preliminary NMR chemical shift data indicate APE1 undergoes a dramatic conformational change upon binding to BCL2. We aim to elucidate this specific interaction and provide insight into the connective roles which APE1 and BCL2 may share in the apoptotic pathway.

50. TMPRSS2, a serine protease expressed in the prostate on the apical surface of luminal epithelial cells and released into semen in prostasomes, is misregulated in prostate cancer cells.

Ya-Wen Chen1,2, Ming-Shyue Lee5, Amanda Lucht1, Feng-Pai Chou1,3, Wei Huang6, Thomas C. Havighurst7, Kyung Mann Kim7, Jehng-Kang Wang8, Toni Antalis1,4, Michael D. Johnson9, and Chen-Yong Lin1,3

Poster Presentation; Room 349; Basic Science C

TMPRSS2, a type II transmembrane serine protease, is highly expressed by the epithelium of the human prostate gland. To explore the regulation and function of TMPRSS2 in the prostate, a panel of monoclonal antibodies with high sensitivity and specificity were generated. Immunodetection showed TMPRSS2 on the apical plasma membrane of the prostate luminal cells, and demonstrated its release into semen as a component of prostasomes, organelle-like vesicles which may facilitate sperm function and enhance male reproduction. In prostate cancer cells, TMPRSS2 expression was increased and the protein mislocalized over the entire tumor cell membrane. In both LNCaP prostate cancer cells and human semen, TMPRSS2 protein was detected predominantly as inactive zymogen forms as part of an array of multiple non-covalent and disulfide-linked complexes, suggesting that TMPRSS2 activity may be regulated by

unconventional mechanisms. Our data suggested that TMPRSS2, an apical surface serine protease, may have a normal role in male reproduction as a component of prostasomes. The aberrant cellular localization, and increased expression of the protease seen in cancer, may contribute to prostate tumorigenesis by providing access of the enzyme to non-physiological substrates and binding-proteins.

51. A structural scaffold in human MutY homolog mediates interactions with the 9-1-1 complex

Paz J. Luncsford, Dau-Yin Chang, Guoli Shi, Jade Bernstein, Dimeka N. Patterson, A-Lien Lu, and Eric A. Toth.

Poster Presentation; Room 349; Basic Science D

MutY homolog (MYH), a DNA glycosylase utilized in base excision repair, removes adenines misincorporated opposite 7,8-dihydro-8-oxoguanine (8-oxoG) thereby preventing mutagenic G:C to T:A transversions. Importantly, biallelic human hMYH mutations cause downstream mutations in tumor suppressors (i.e. APC) and proto-oncogenes (i.e. K-ras) and are implicated in the development of 1-3% of colorectal cancer cases. Some hMYH variants are defective enzymes because of decreased binding affinity to their DNA substrate; however, other hMYH variants likely promote oncogenesis because of impaired interactions with proteins, such as the 9-1-1 complex, which mediates the cell-cycle checkpoint response. Here we report the first eukaryotic structure of a MutY protein, the catalytic domain of hMYH (residues 65-350), to 2.2 Å. The linker region between the catalytic- and C-terminal- domains of hMYH differs from its bacterial counterpart existing in an extended conformation that would provide an ideal scaffold for 9-1-1 to dock. Through creation of an S. pombe chimera construct with an E. coli linker domain, we demonstrate that, although the SpMyh1 linker region is not needed for DNA-binding, it is required for robust enzymatic activity. We elucidate that residues I261 and E262 of SpMyh1 are required for its interaction with Hus1 of the 9-1-1 complex. The SpMyh1(I261A/E262Q) mutant cannot complement the mutator phenotype of myh1Δ cells and interruption of the SpMyh1/Hus1 interaction increases cell sensitivity to H2O2. Our results suggest that the MYH/9-1-1 interaction is an important step for DNA repair and, disruption of this interaction may contribute to the development of colorectal cancer in some patients.

52. Quantum Dot Based Artificial Antigen Presenting Cell Activation of Natural Killer T Cells

Carolyn Morris, Danubia Hester, Wenji Sun, Wei Lin, Tonya J. Webb

Poster Presentation; Room 349; Basic Science A

Natural killer T (NKT) cells are a unique subset of T cells that display markers characteristic of both natural killer (NK) and T cells. NKT cells have been shown to be important in regulating immune responses to tumors. To date, NKT cell based immunotherapy has been limited by the use of autologous dendritic cells. The function of dendritic cells can vary substantially from person to person. Consequently, our laboratory has developed bead based artificial antigen presenting cells (aAPC) to activate and expand NKT cells. We have found that these aAPC facilitate the ex vivo

expansion of primary NKT cells but, due to their relatively large 4.5mm size, have limited in vivo application. Therefore, in these studies we sought to generate smaller aAPC using nanotechnology. Nanobiotechnology is focused on the development of nanoscaled materials and devices for use in drug delivery, imaging, and cell tracking. The hypothesis to be tested in this project is that nanotechnology can be used to assess NKT cell function and modulate their anti-tumor responses. Utilizing recent advances in nanotechnology, we have generated CD1d-Ig based aAPC and Qdot-APC, made by covalent coupling of CD1d-Ig and costimulatory molecules to magnetic beads or quantum dots, respectively. Quantum dots are 10-20 nm nanoparticles that are inherently fluorescent. They are made of semiconductor materials coated with an amphiphlic polymer which allows the nanoparticles to be functionally modified. We have found that smaller Q-dot aAPC can effectively stimulate NKT cells. Thus, the bead based aAPC and Qdot-APC can be used as a standardized method for the stimulation and expansion of NKT cells, and Qdot-APC have potential in vivo applications, such as cancer immunotherapy.

53. Short-term stimulation of cultured skeletal muscle fibers alters Glut4 transporter trafficking in response to insulin

Patrick Robison, Martin F. Schneider

Poster Presentation; Room 349; Basic Science B

Failure to activate the facilitated glucose transporter Glut4 in response to insulin is responsible for initial pathogenesis of diabetes, which currently affects 220 million people worldwide. The inactive transporter is sequestered in intracellular membranes in deep intracellular stores. In response to either insulin or exercise (metabolic demand) Glut4 containing vesicles must be trafficked to and inserted into the outer membranes where the transporter can access extracellular glucose. Sensitivity of the transporter to insulin and overall glucose homeostasis are known to improve with long-term exercise of the skeletal muscles. Paradoxically, in this study we have observed that the internal Glut4 stores of cultured fast-twitch skeletal muscle fibers subjected to short term (4hrs) repetitive stimulation (10 Hz x 5sec/50sec) have a diminished sensitivity to insulin. We have also observed concurrent rearrangement of cytoskeletal elements which may account for this effect. Specifically, the tubulin cytoskeleton of the stimulated fibers collapses into a narrow network near the sarcolemma. The tubulin cytoskeleton is known to have different configurations in fast and slow twitch muscle, with fast type fiber tubulin networks showing deep invasions into the core of the fiber and slow type fibers showing a narrow tubulin network near the sarcolemma. Additionally, fiber stimulation at this frequency has been shown to activate fiber type specific transcription factors in culture, although the timescale of our experiments is not long enough for fiber type transformation to complete. We believe our data indicate an early shift from the fast fiber type toward the slow fiber type tubulin distribution. We propose a mechanism whereby cytoskeletal rearrangement in response to exercise orphans internal stores transiently to account for this result.

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