33rd Annual Graduate Research Conference

33rd Annual UMB Graduate Research Conference

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Foreword by GSA Executive Board

Welcome to the 33rd 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 Center for Research on Aging (ORC) and 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, will offer the second 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 thank our keynote speaker, Dr. Neal Fedarko, a senior faculty member of Geriatric Medicine, Gerontology, and Translational Research at Johns Hopkins University, 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.

33rd Annual Graduate Research

Conference Keynote Speaker

Dr. Neal Fedarko

Senior faculty member of Geriatric Medicine, Gerontology, & Translational Research

Johns Hopkins University

Dr. Fedarko received an A.B. in Chemistry from Oberlin College in 1980 and a Ph.D. in Biochemistry from the University of Illinois at Champaign-Urbana in 1987. After post-doctoral work at the National Institutes of Health, he was recruited to Johns Hopkins University in 1992 where he is currently a Professor in the Division of Geriatric of Medicine and Gerontology, Director of the Translational Research Training Program in Gerontology & Geriatrics, Director of the Clinical Research Unit Core Laboratory in the Institute for Clinical and Translational Research, and Co-director of the Biology of Frailty Program. Dr. Fedarko's research interests include age-related dysregulation of tissue remodeling, cellular differentiation and apoptosis, with an emphasis on tumor progression.

SCHEDULE OF EVENTS

33rd Annual Graduate Research Conference
Thursday, April 7, 2011
University of Maryland, Baltimore

8:00 - 9:00am	**BREAKFAST & REGISTRATION**
9:00am – 12:00pm	**POSTER PRESENTATIONS**
9:00-10:30	Basic Sciences Poster Sessions 3 (Room 349)
9:00-10:30	Basic Sciences Poster Sessions 4 (Room 349)
9:00-10:30	Informatics/Social Sciences/Policy Poster Sessions 1 (Room 349)
10:30-12:00	Basic Sciences Poster Sessions 5 (Room 349)
10:30-12:00	Basic Sciences Poster Sessions 6 (Room 349)
10:30-12:00	Informatics/Social Sciences/Policy Poster Sessions 2 (Room 349)
9:00am - 12:30pm	**ORAL PRESENTATIONS**
	Basic Sciences Oral Session 1 (Room 353)
	Basic Sciences Oral Session 2 (Room 351)
12:30 - 2:00pm	**LUNCH & KEYNOTE ADDRESS** (Ballroom)
	Dr. Neal Fedarko
	Senior Faculty, Johns Hopkins University
2:00pm	**AWARDS** (Ballroom)

Abstracts

1. Staphylococcus aureus Nasal Colonization and the Host Immune Response

Nate Archer, Dr. Mark Shirtliff

Oral Presentation; Room 353; Basic Science 1

Background: Staphylococcus aureus is the etiological agent of a myriad of human diseases and is increasingly linked to antimicrobial resistance. The ecological niche of S. aureus in humans is the anterior nares where either persistent or intermittent colonization occurs. A strong link has been discovered between S. aureus nasal carriage and S. aureus nosocomial infection with persistently colonized patients at greater risk for infection as compared to the intermittently colonized. Despite this knowledge, little is known about the immune response to S. aureus nasal carriage. Our research begins to define this response, in terms of cellular, cytokine, and antibody components, using a mouse model of S. aureus nasal colonization. Methods: C57BL/6J, SCID, and Rag-1KO mice were inoculated intra-nasally with 10^8 CFU of a clinical isolate of S. aureus. At several time points post-inoculation the mice were bled and nasal tissues were harvested and homogenized. Tissue homogenates were used to enumerate CFU/nose counts and colonization rates with S. aureus-CHROMagar plates. In addition, various cytokines were measured in these homogenates by multiplex assay. Immunogenic S. aureus cell wall proteins recognized by serum IgA were visualized by Western blot and ELISA assays, and responses were compared at various time points post-inoculation. In addition, immunogenic cell wall proteins were identified by MALDI-TOF analysis. Results: A statistically significant increase in CFU/nose and colonization rate was observed at 28 days post-inoculation in both SCID and Rag-1KO as compared to wild-type C57BL/6J mice. The cytokines IL-1β and IL-17 were significantly up-regulated at 2 and 7 days post-inoculation in wild type mice, respectively, as compared to un-colonized controls. Levels of IgA antibodies against S. aureus proteins increased significantly at 7 days post-inoculation as compared to control serum. Lastly, S. aureus protein A was identified as an immunogenic protein recognized by IgA.

Conclusions: Evidence provided by SCID and Rag-1KO mice suggests that B and/or T cells are important for clearance of S. aureus from the nasal mucosa. In addition, IL-1² and IL-17 may have a role in prompting de-colonization of S. aureus by the immune response. Finally, S. aureus protein A is targeted by host IgA and may have implications as a vaccine candidate.

2. The Role of Semaphorin 4D and Plexin-B1 in Perineural Invasion

Nada O. Binmadi, Hua Zhou, Patrizia Proia, Yinghua Yang, Yi-Ling Lin and John R. Basile

Oral Presentation; Room 353; Basic Science 1

Perineural invasion (PNI) is a tropism of tumor cells for nerve bundles in the surrounding stroma. It is a unique pathological feature observed in certain tumors, referred to as neurotropic malignancies, which severely limits the ability to establish local control of disease and results in pain, recurrent growth and eventual distant metastases. Despite its importance as a prognostic indicator, the biology of PNI is poorly understood and as a result, no treatment modalities have been developed to address this aspect of tumor spread. The semaphorins and their receptors, the plexins, are a family of proteins originally shown to be expressed in nerves that are important in nerve cell adhesion, axon migration and proper central nervous system development. Emerging

evidence has demonstrated that these factors are expressed in many tissues outside of the nervous system and represent a widespread signaling system that is involved in regulation of motility and adhesion in different cell types. We believe that the plexins and semaphorins, which are strongly expressed in both axons and many carcinomas, play a role in PNI. Here we show that Plexin-B1 is overexpressed in many tissues and cell lines from neurotropic malignancies and is attracted to nerves expressing its ligand, Semaphorin 4D (Sema4D). We also demonstrate that nerves are attracted to tumors through this same system of proteins, suggesting that Plexin-B1 and Sema4D are important in the promotion of PNI.

3. FOOTSHOCK INDUCED HABENULAR CFOS DIMINISHED FOLLOWING LESION OF THE FASCICULUS RETROFLEXUS

P. Leon Brown

Oral Presentation; Room 353; Basic Science 1

Midbrain dopaminergic neurons are an essential part of the neurocircuitry of reinforcement. They are activated by rewards or reward-predicting cues and are inhibited by reward omission. The lateral habenula (LHb), an epithalamic structure that forms reciprocal connections with midbrain dopaminergic neurons via the fasciculus retroflexus (FR), shows an opposing response, being activated by reward omission or aversive stimuli and inhibited by reward-predicting cues. Electrical stimulation of the LHb inhibits dopaminergic neurons and this effect is blocked by lesions of the FR, providing evidence of a functional relation between these areas. However, the functional relevance of dopaminergic connections to the LHb is unclear. Here we offer preliminary evidence that an aversive stimulus, footshock, induces cFos activation in the LHb that is dopamine dependent. Expression of cFos in the LHb was higher in footshocked rats than in naive rats. A lesion of the FR diminished this effect. The paraventricular nucleus of the thalamus (PVThal), a region which shows cFos expression after footshock but which has no direct connections through the FR, also showed elevated cFos expression in shocked rats but with no effect of lesion. Furthermore, the majority of cFos positive cells in the LHb were concentrated in the medial subnuclei, an area that receives dense dopaminergic innervation. These data suggest that dopaminergic input may modulate footshock induced cFos expression in the LHb. Future work will investigate the effect of dopamine specific neuronal lesions on cFos expression in the LHb.

4. THE HSV-2 ONCOLYTIC VIRUS ΔPK INDUCES MULTIPLE DEATH AND INFLAMMATORY PROGRAMS ASSOCIATED WITH INHIBITION OF MELANOMA TUMOR GROWTH.

Aric Colunga, Jennifer Laing, Laure Aurelian

Oral Presentation; Room 353; Basic Science 1

Metastatic melanoma is a highly aggressive and drug resistant cancer. Oncolytic virotherapy is a novel therapeutic strategy designed to circumvent cancer resistance through virus replication-induced cancer cell lysis. Unfortunately, its clinical efficacy is modest, apparently related to poor virus replication within the tumors and the failure to stimulate inflammatory/immunotherapeutic pathways. The growth compromised herpes simplex virus type 2 (HSV-2) mutant ΔPK causes metastatic melanoma cell death through activation of multiple non-redundant death pathways. Activation of calpain and caspases-3 and -7 provide the bulk of the death programs, as evidenced by the finding of 80% protection in cells treated with the combination of calpain (PD150606) and pancaspase (zVAD-fmk) inhibitors. In

addition, ΔPK upregulates the autophagy proteins Beclin-1 and LC-II and the autophagy inhibitor 3-methyladenine (3-MA) reduces ΔPK-induced cell death by 15-17%. Cell death is further decreased (to a total of 28-34%) by treatment with 3-MA together with the JNK inhibitor SP600125, indicating that JNK signaling and autophagy contribute independently to ΔPK-induced cell death. Inflammatory-related pathways contribute to ΔPK-induced melanoma cell death, as evidenced by caspase-1 activation, interleukin (IL)-1β release into the conditioned medium and upregulation of pro-apoptotic/stress signaling molecules and pro-inflammatory cytokines and chemokines identified by microarray analysis. These include BAD, BIK, BOK, NLRC4, CIDEB, IL-1β, IL-6, IL-8, IL-12α, IL-12β , LTα, CXCL-10, c-JUN and caspases-4, -5, -6, -8, and -10. Tumor burden is significantly reduced by intratumoral injection of ΔPK and xenografts generated from a fresh clinical isolate, were completely eliminated for a period of 1 year following treatment. Growth inhibition is associated with activation of calpain and caspases-3, -7 and -1 and expression of the inflammatory cytokine TNF-α. Collectively, the data indicate that ΔPK eliminates tumor burden through the simultaneous activation of multiple non-redundant death programs and pro-inflammatory signaling, providing a distinct and promising virotherapeutic approach.

5. THE D299G POLYMORPHISM INHIBITS TLR4 SIGNAL TRANSDUCTION BY INTERFERING IN THE MYD88- AND TRIF-DEPENDENT PATHWAYS

Leandra Figueroa and Andrei E. Medvedev, PhD

Oral Presentation; Room 353; Basic Science 1

Toll-like Receptor 4 (TLR4) senses Gram-negative bacterial lipopolysaccharide (LPS) to activate expression of pro-inflammatory cytokines and type I interferons (IFN) via the myeloid differentiation primary response gene (MyD88)- and TIR-domain containing adapter-inducing interferon-β (Trif)-dependent pathways, respectively. The Asp299Gly (D299G) and Thr399Ile (T399I) polymorphisms in TLR4 have been associated with increased risk of certain bacterial infections, Gram-negative sepsis, and tuberculosis. However, the molecular mechanisms by which these polymorphisms affect TLR4 signaling remain poorly understood. In this study, we sought to determine the impact of the D299G and T399I TLR4 polymorphisms on LPS binding and LPS-elicited activation of the MyD88- and Trif-dependent pathways. To measure LPS binding, 293T/MD2/CD14 transfectants expressing WT or mutant YFP-TLR4s were incubated with biotinylated LPS, followed by staining with streptavidin-allophycocyanin (SA-APC). FACS analyses showed comparable LPS binding to cells expressing wild-type (WT) or D299G YFP-TLR4 species. Transfection-based complementation of TLR4-deficient HEK293 with CD14, MD2, and WT vs. mutant YFP-TLR4 variants revealed decreased LPS-elicited phosphorylation of TBK1 and p38, activation of NF-κB and IFN-β luciferase reporters, and expression of IL-8 and INF-β mRNA in cells expressing the D299G variant, in contrast to strong responses triggered by WT TLR4. Western blot analyses indicated comparable protein levels of WT and mutant YFP-TLR4, Flag-MD2, total p38, total TBK1, and β-tubulin. Our data indicate that the D299G polymorphism compromises the ability of TLR4 to activate MyD88- and TRIF-dependent pathways but does not affect LPS binding to cell transfectants.

6. The Role of Adaptive Immunity in the Protection Confered by a Francisella lipid A Mutant

Daniel A. Powell, Duangjit Kanistanon, Adeline M. Hajjar, Mark R. Pelletier, Ilana E. Cohen, Sing Sing Way, Shawn J. Skerrett, Xiaoyuan Wang, Christian R.H. Raetz and Robert K. Ernst

Oral Presentation; Room 353; Basic Science 1

Francisella tularensis subspecies tularensis (Ft) is an intracellular Gram-negative bacterium and the causative agent of the severe human disease tularemia. Francisella lipid A, normally the biologically active component of lipopolysaccharide (LPS) has no to low endotoxic activity. A Francisella tularensis subspecies novicida (Fn) lipid A biosynthesis mutant was generated that lacked the 4´-phosphatase enzyme (LpxF). Analysis of lipid A isolated from this mutant strain showed the retention the phosphate moiety at the 4´ position and the N-linked fatty acid at the 3´ position on the diglucosamine backbone. This mutant was previously shown to be avirulent in the footpad infection model. Studies were undertaken to determine if this mutant was avirulent by more natural routes (aerosol and subcutaneous) of infection and to determine if this mutant could provide protection from a lethal WT challenge. All mice (C57BL/6) infected with the lpxF-null Fn mutant either by subcutaneous or the pulmonary route survived initial infection and subsequently developed protective immunity against a lethal wild type challenge.
The source of this protective immunity was further elucidated in a number of ways. Firstly mice were depleted of either CD4 or CD8 T cells before a lethal WT challenge. ~50% of mice depleted of CD4 T cells succumbed to the challenge, where as all mice depleted of CD8 T cells survived the challenge, these results demonstrate a partial dependence on CD4 T cells while CD8 T cells are dispensable for protection. Secondly B-cell deficient ¼MT mice were primed with the lpxF-null Fn mutant and then challenged with a lethal WT Fn dose. All ¼MT mice succumbed to lethal challenge indicating the protection was dependent on antibody production.
This work was supported by a grant from the National Institutes of Health (1U54 AI57141).

7. The second postnatal week is a sensitive period for normal cerebellar Purkinje cell development through the PGE2-E2 pathway in the rat

Jessica F. Knutson, Margaret M. McCarthy

Oral Presentation; Room 351; Basic Science 2

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. Prostaglandin E2 (PGE2) is a key regulator of fever following inflammation throughout the central nervous system, and 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 PGE2. Previously we have shown that during the second week of life in Sprague Dawley rats PGE2 inhibits growth of cerebellar Purkinje cell dendrite length and spine number. This occurs through PGE2 s activation of the enzyme aromatase, which converts testosterone to estradiol. Systemic treatment and direct injection into the cerebellum with estradiol or PGE2, respectively, have similar effects in decreasing Purkinje cell arborization. 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. Reduction of PGE2 synthesis by COX1/2 inhibitors has the opposite effect, increasing Purkinje cell arborization. In addition to the

immediate effects of PGE2 on Purkinje cell morphology, long-term effects are seen in behavior. Normally, males engage in more frequent social play behavior than females. Males treated with the COX-inhibitor, nimesulide, have reduced social play behavior, perseverative object exploratory behavior, and a heightened somatosensory threshold. These sexually-dimorphic changes in behavior are parallel to symptoms in children with autism spectrum disorder (ASD). The exact cause is unknown, but ASD is currently viewed as a neurodevelopmental disorder with complex genetic and environmental origins. Cerebellar pathology is strongly associated with ASD, as is inflammation during the developmental period between in utero and early childhood. We have shown that there is a sensitive period where effects of PGE2 are only seen during the second postnatal week. This developmental window may put children at higher risk of developmental diseases such as ASD from exposure to fever/inflammation and over-the-counter fever reducers.

8. THE ROLE OF THE FRANCISELLA TULARENSIS PHT SUBFAMILY OF MFS TRANSPORTERS IN INTRACELLULAR SURVIVAL AND VIRULENCE

Mark E. Marohn, Araceli Santiago, and Eileen M. Barry

Oral Presentation; Room 351; Basic Science 2

Francisella tularensis is a CDC category A select agent and the causative agent of tularemia. Because it is aerosolizable and has a low infectious dose, F. tularensis poses a significant threat as a potential bioterror agent and is a priority for vaccine development. The ability of F. tularensis to survive and replicate in macrophages and other cell types is a critical component of its pathogenesis, and the inability to survive and replicate in macrophages has been linked to in vivo attenuation. The F. tularensis genome contains a family of nine genes encoding a subfamily of Major Facilitator Superfamily (MFS) transporters, which have homology to factors that have been linked to the ability to replicate in macrophages in other intracellular pathogens. Our hypothesis is that these MFS transporters will play a role in the intracellular survival and replication of F. tularensis and in host response to infection and will serve as viable targets for attenuation and vaccine development. Mutants containing deletions in eight unique genes in the MFS family have been constructed in the F. tularensis LVS background and these mutants have been evaluated for changes in a variety of phenotypic assays using murine macrophages and HepG2 human hepatic cells. Three LVS MFS mutant strains have been demonstrated to be defective in intracellular replication in HepG2 cells compared to parental LVS, with a similar defect observed in two of these mutants in both J774 murine macrophages and murine thioglycollate-elicited peritoneal macrophages. Interestingly, while these mutants show a defect in replication early in an infection, they replicate more efficiently than wild type later in the infection and are recovered at near wild type levels. We hypothesize that this phenotype is due to altered expression of the remaining MFS transporters during infection or delayed phagosomal escape. These mutant strains are attenuated for virulence in BALB/c mice and protective against a subsequent lethal challenge with LVS. We observed altered patterns of organ dissemination in BALB/c mouse infections with these mutants in comparison to parental LVS. To further characterize the observed mutant phenotypes, continuing work includes examining the expression kinetics of all Pht subfamily MFS genes during intracellular infections, phagosomal escape kinetics, and macrophage and tissue-specific cytokine responses to MFS mutant infection. We have shown that MFS transporters are involved in the pathogenesis of F. tularensis, and we believe that further

understanding of these mutant strains will continue to shed light on the pathogenic mechanisms of F. tularensis and prove these genes to be viable targets for the development of live attenuated vaccines.

9. IDENTIFICATION OF NEW GENUS-SPECIFIC TYPE III SECRETION EFFECTORS OF CHLAMYDIA SPP.

Sergio Mojica, Kelley Hovis, Laura Pedersen, Jason McDermott and Patrik Bavoil

Oral Presentation; Room 351; Basic Science 2

The chlamydial type-III-secretion (T3S) system allows for translocation of specific effector proteins into the host cell cytosol that target host proteins in order to facilitate pathogenesis and create a more permissive intracellular environment. The genetically intractability of Chlamydia and limited sequence similarity to known T3S effector proteins of other Gram negative pathogens has made it difficult to identify new T3S effectors in Chlamydia. A Chlamydia-specific derivative of the SIEVE algorithm termed cSIEVE was developed by training SIEVE with known chlamydial effector sequences. cSIEVE predicted effector candidates from various Chlamydia species were then tested in the heterologous T3S system of Y. pseudotuberculosis. Two new effectors were identified: ORF-062 encoding a 45 kDa protein in C. psittaci strain CAL10, and CT049 encoding a 50 kDa protein in C. trachomatis serovar E. ORF-062 is at the same genomic locus as the previously described C. trachomatis effector CT694. Interestingly, the two proteins share limited sequence similarity, indicating possibly divergent functions. CT049 is notorious in that it encodes a previously described protein named Pls1 with sequence similarity to the passenger domain of the autotransported polymorphic membrane protein PmpC. The presence of orthologs in all other chlamydial genomes suggests that they may be important in steps of pathogenesis and development that are shared between species. Subsequent studies will attempt to identify molecular and subcellular targets of these effectors in infected cells, thereby promoting further understanding of chlamydial pathogenesis.

10. ZOLEDRONIC ACID REVERSES THE EPITHELIAL-MESENCHYMAL TRANSITION WHILE INHIBITING THE CANCER STEN CELL POPULATION OF HIGHLY TUMORIGENIC BREAST CANCER CELL LINES

Amanda J. Schech, Rabia Gilani, Armina A. Kazi, and Angela H. Brodie

Oral Presentation; Room 351; Basic Science 2

Breast cancer remains the second leading cause of cancer related death amongst women in the United States. This is largely due to metastasis of cancer cells from the primary tumor to other parts of the body, and to the putative cancer stem cell population. Both are driven by the epithelial-mesenchymal transition (EMT), a cellular process whereby cancer cells of epithelial origin lose their epithelial characteristics and acquire a mesenchymal phenotype. Cells which undergo EMT tend to be motile and invasive, and therefore can metastasize to other parts of the body. EMT has also been implicated in the generation of cells expressing the cancer stem cell phenotype. As metastatic disease and the cancer stem cell are increasingly difficult to treat, advances in pharmacotherapy are required. Zoledronic acid, originally indicated for use in the treatment of osteoporosis, has been reported to inhibit the growth of cancer cells. The mechanism of this effect however has yet to be determined. Preliminary data suggests that treatment with zoledronic acid reduces expression of cyclooxygenase-2, an established regulator of EMT. Based on these findings, it was hypothesized that treatment with zoledronic acid can reverse

EMT in cancer cells, driving them to express a more epithelial phenotype. To test this hypothesis, the highly metastatic, triple negative breast cancer cell lines MDA-MB-231 and Hs578t, which largely express mesenchymal characteristics, were used to measure cell viability and changes in protein expression following treatment with zoledronic acid. Dose response analysis showed an IC50 of approximately 2μM in the Hs578t and 6 μM in the MDA-MB-231 cell line. Zoledronic acid treated cells displayed a decreased mesenchymal phenotype, as evidenced by decreased expression of mesenchymal markers N-cadherin and TWIST. This was accompanied by a subsequent increase in epithelial phenotype as evidenced by increased expression of epithelial marker E-cadherin. To further elucidate the effects on the mesenchymal and epithelial phenotypes of these cells, surface expression of CD24 and CD44 were measured by flow cytometry. While vehicle treated samples of both cell lines stained positive for CD44 (mesenchymal marker) and negative for CD24 (epithelial marker), zoledronic acid treatment increased CD24 expression, corroborating earlier findings. As an increased ratio of surface expression of CD44+/CD24- and mammosphere formation are characteristic of the breast cancer stem cell, effects of zoledronic acid on this subpopulation in Hs578t cells were determined. Cells pretreated with zoledronic acid for 72 hours were seeded under mammosphere conditions and allowed to propagate for 7 days. Zoledronic acid treated cells formed significantly reduced mammospheres, while the ones that formed were reduced in size. These findings suggest that zoledronic acid is able to reverse the epithelial mesenchymal transition, which may reduce the tumor initiating capacity of highly metastatic cells.

11. MULTIPLE RNA INTERACTION MODES OF AUF1

Zucconi, B.E.; Ballin, J.D.; Wilson, G.M.

Oral Presentation; Room 351; Basic Science 2

The translational potential of an mRNA hinges upon its cytoplasmic concentration which is a function of both its rate of synthesis and rate of decay. One factor that can guide the cytoplasmic turnover of specific mRNAs is the presence of an AU-rich element (ARE) in its 3'-untranslated region. AREs are highly diverse but commonly contain one or more AUUUA pentamers in a U-rich region. AUF1 is an RNA-binding protein that binds AREs with high affinity and regulates the stability of mRNAs containing these motifs. Our previous work demonstrated that AUF1 proteins are dimers in solution, and form oligomeric structures on RNA substrates. The three-dimensional structure of an mRNA may occlude or expose binding sites for many RNA-binding proteins or microRNAs which may dictate mRNA fate. Therefore, the nucleotide span length and identity covered by AUF1 and the induced structure of the adjacent RNA sequence becomes pivotal. In this study we provide evidence that AUF1 dimers may remodel local RNA structure by recognizing two disparate sequence elements in RNA substrates. First, macromolecular binding density analysis (MBDA) showed that optimal AUF1 binding to RNA requires a site size of 34 nucleotides, over twice that expected when compared to structures of similar RNA binding proteins. Second, global fits of AUF1 binding isotherms across varying concentrations of RNA showed significant deviation from a simple sequential dimer binding model, particularly at high concentrations of RNA and low concentrations of AUF1. The addition of parameters representing an independent RNA-binding interface to our AUF1 binding model resolved the deviations of the data from the global fit analysis. Finally, we

show that AUF1 can bind RNA substrates containing small, separated U-rich domains with affinity comparable to a 38-nucleotide contiguous substrate. Together, these alternative modes of AUF1-mRNA interaction indicate that AUF1 may interact with a wide variety of cellular RNA substrates and provides a biochemical rationale for the local RNA remodeling activity of this protein.

12. Fn14 Receptor Levels Regulate Glioma Cell Invasiveness

Emily Cheng and Jeff Winkles

Poster Presentation; Room 349; Basic Science 3

Fibroblast growth factor-inducible 14 (Fn14) is a member of the tumor necrosis factor (TNF) receptor superfamily. The only known ligand for this receptor is the cytokine TNF-like weak inducer of apoptosis (TWEAK). Studies to date indicate that Fn14 signaling can be activated via two mechanisms: (1) TWEAK binding to the Fn14 extracellular domain, and (2) Fn14 overexpression. Both TWEAK and Fn14 are present in several solid tumor types, and research is currently being done to elucidate whether this signaling axis may affect cancer progression. Glioblastoma multiforme (GBM) is the most lethal form of brain cancer, with a median survival of about one year. These tumors express multiple angiogenic factors and vascularization is critical for tumor growth. In addition, glioma cells are highly invasive and this makes it difficult to completely resect or effectively treat brain tumors with chemotherapy or radiotherapy. Previous immunohistochemical studies have shown that Fn14 gene expression is upregulated in glioma cells in both the tumor core and invasive rim regions of GBM specimens. High Fn14 levels in GBM tumors positively correlate with poor prognosis. In contrast, TWEAK expression is low in advanced glioma tumors. Thus, it is thought that TWEAK-independent Fn14 signaling may occur in glioma cells in vivo. In support of this, previous studies have indicated that Fn14 overexpression in transiently transfected glioma cells activates the NF-κB signaling pathway and promotes invasion. Furthermore, when treated with the chemotherapeutic, camptothecin, these cells exhibit an increased survival capacity. These responses do not occur if an Fn14 mutant with a truncated cytoplasmic tail is expressed in these cells. Other studies have shown that transient Fn14 knockdown in glioma cells using siRNA duplexes reduces cell invasion. We are generating stable glioma cell lines with either elevated or reduced Fn14 levels in order to study these effects in more detail, and to conduct long-term in vivo growth and invasion assays. Here, we show that stable overexpression of Fn14 has no significant effect on glioma cell growth, but increases glioma cell invasiveness. In addition, stable knockdown of Fn14 using shRNA decreases glioma cell invasiveness. Further studies are planned to determine what genes and signaling pathways are regulated by TWEAK-independent Fn14 signaling, and how they may affect invasion, and chemoresistance in glioma cells.

13. Inhibition of Matriptase by Extravascular Antithrombin in Epithelial Cells but Not in Most Carcinoma Cells

Feng-Pai Chou, Han Xu, Ming-Shyue Lee, Ya-Wen Chen, Richard Swanson, Steve T. Olson, Michael D. Johnson, and Chen-Yong Lin

Poster Presentation; Room 349; Basic Science 3

Antithrombin, a major anticoagulant, is robustly transported into extravascular compartments in which its target proteases are largely not characterized. The serpin was previously detected in human milk as complexes with matriptase, a membrane-

bound serine protease broadly expressed in epithelial and carcinoma cells and under tight regulation by hepatocyte growth factor activator inhibitor (HAI)-1, a transmembrane Kunitz-type serine protease inhibitor that forms heat-sensitive complexes with matriptase. When matriptase zymogen activation is induced in mammary epithelial cells, active matriptase is rapidly inactivated by forming complexes with HAI-1, as expected. A proportion of active matriptase, in the current study, was unexpectedly detected in heat-resistant complexes, likely with serpin-type protease inhibitors. Antithrombin was detected among these matriptase complexes. Matriptase-antithrombin complexes were also detected in other non-tumorigenic mammary and prostate epithelial lines, suggesting that in addition to HAI-1, matriptase in epithelial cells is under the control of interstitial antithrombin. This physiological mechanism, however, appears largely lost in cancer cells as matriptase-antithrombin complexes were not detected in seven cancer cells with breast, prostate and ovarian origins, when mass matriptase activation was induced in these cells. By using active matriptase isolated from multiple myeloma cells, we further demonstrate the formation of matriptase-antithrombin complex and that heparin can significantly potentiate the inhibitory potency of antithrombin against matriptase. Second order rate constants for the inhibition were determined to be 3.88x103 M-1sec-1 in the absence of heparin, and 9.57x104 M-1sec-1 in the presence of heparin, a 25-fold increase and consistent with the role of heparin in antithrombin activity. Taken together these data suggest that normal epithelial cells employ a dual mechanism with HAI-1 and antithrombin to control matriptase and that the antithrombin-based mechanism appears lost in majority of carcinoma cells.

14. Development Of A Coarse-Grained Model For The Surfactant Family of Linear Alkylbenzene Sulfonates

Xibing He, Wataru Shinoda, Russell DeVane, Olgun Guvench, Kelly L. Anderson, Alexander D. MacKerell Jr. , Michael L. Klein

Poster Presentation; Room 349; Basic Science 3

A coarse-grained (CG) model has been developed for the anionic surfactant class, linear alkylbenzene sulfonates (LAS), which are the most widely used synthetic surfactants. The development work started from a systematic examination of tens of CG water models with different resolutions, interaction potentials (Lennard-Jones and Morse), and cut-off distances. The relationships between the parameters under specific choices of the above options and the thermodynamic properties, such as density, surface tension, and compressibility, were found to fit simple mathematical equations. The limits of applicability of these CG water models were explored by checking the melting temperature. Considering both efficiency and accuracy, a CG water model which includes three water molecules in one CG site was chosen. Correspondingly, the LAS molecules were mapped into CG sites each contains approximately three heavy atoms and connected hydrogens. Structural data obtained from atomistic simulations and thermodynamic data from experiments were used as targets to parameterize standard potential forms for bonded and non-bonded interactions. An extensive evaluation of the CG model for a series of different alkane molecules (aliphatic or aromatic, linear or branched) shows that the present model is not only reliable, but also transferable. This point is crucial to assure that the model is capable of representing different isomers and homologues in the LAS family. The resulting model is easily implemented into standard MD codes. The added computational efficiency permits the

simulation of the self-assembly of LAS solutions starting from a random configuration. The model is shown to accurately reproduce the phase behavior of solutions of pure isomers of sodium dodecylbenzene sulfonate, despite the fact that phase behavior was not directly taken into account in the parameterization.

15. LOCALIZATION OF OBSCURIN KINASES IN THE EXTRACELLULAR MATRIX OF STRIATED MUSCLE CELLS.

Li-Yen Hu, Jane Valenti, and Aikaterini Kontrogianni-Konstantopoulos

Poster Presentation; Room 349; Basic Science 3

Obscurins are giant muscle proteins implicated in myofibrillogenesis and linked to hypertrophic cardiomyopathy. They contain a tandem array of adhesion, i.e. immunoglobulin (Ig) and fibronectin-III (Fn-III) domains, and 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) motif, and two serine/threonine kinase domains, namely SK1 and SK2. Three out of the four reported obscurin isoforms contain single or tandem kinase domains. Using immunofluorescence combined with confocal microscopy and antibodies specific to the kinase-bearing obscurin isoforms, we found that these are present in diverse locations in striated muscle cells, including the sarcolemma, the Z/I junction and the M-band. Our 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 revealed that the extracellular domain of the β²-subunit of Na+/K+-ATPase (NKA) 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. Given the extracellular localization of the interacting portions of NKA and N-cadherin, we next examined their co-distribution with SK1 and SK2, as a means of validating their physiological binding in the context of the cell. To this end, we used non-permeabilized primary cultures of skeletal and cardiac muscle cells and labeled them with antibodies to NKA, N-cadherin and the kinase domains of obscurin. Our results demonstrated that at least some portions of the obscurin-kinase isoform(s) are localized extracellularly. Currently, we are investigating the roles of SK1 and SK2 in regulating cardiac function by manipulating their expression levels through adenoviral-mediated gene delivery.

16. ANTAGONISM OF THE BAK-BCL-XL COMPLEX BY SYNTHETIC α-HELIX MIMETICS OF VARYING BACKBONE CURVATURES

Jeremy L. Yap, Kenno Vanommeslaeghe, Alexander D. MacKerell Jr., Steven Fletcher

Poster Presentation; Room 349; Basic Science 3

The discovery of potent small molecule inhibitors of protein protein interactions (PPIs), which play pivotal roles in cells, remains a challenging goal. Large interfacial areas and often non-contiguous contact points are but a couple of the features that have deterred medicinal chemists from tackling these important therapeutic targets. Despite these issues, there have been many success stories owing largely to the identification of hot spots , regions at the PPI interface that contribute significantly to the stabilization of the complex. One such protein protein complex of interest is Bak Bcl-xL, whose antagonism through binding the Bak-binding domain on the surface of Bcl-xL has been validated as an anti-cancer therapeutic strategy. A hot spot of this PPI is the interaction of the hydrophobic face of the BH3 α-helix of Bak (a pro-apoptotic

protein) with a hydrophobic crevice on Bcl-xL (an anti-apoptotic protein). Reminiscent of a cavity within an enzymes' active site, this crevice poses an attractive target for drug design. Previous research has identified a synthetic α -helix mimetic of the Bak-BH3 helix based on an oligoamide-foldamer strategy, which disrupts the Bak Bcl-xL interaction with an IC50 value of 5 μM. The heterocyclic nitrogens of the four pyridine subunits in this tetrameric oligoamide engage in intramolecular, bifurcated hydrogen bonds, leading to a rigid structure and an induction of backbone curvature. We have stepwise replaced the pyridine rings with benzene rings to afford more flexible and less curved α -helix mimetics more akin to the native Bak-BH3 helix, and we will present our findings.

17. Intermediate Filaments Affect Microtubule Organization and Mitochondrial Trafficking in Skeletal Muscle Fibers

Jaclyn P Kerr, Andy P Ziman, Andrea O'Neill, Michael P Kiley, Stuart S Martin, Robert J Bloch

Poster Presentation; Room 349; Basic Science 3

The cytoskeleton of striated muscle is a highly regular, organized network comprised of actin microfilaments, intermediate filaments (IF), and microtubules (MT). The absence of the IFs has profound effects on membrane organization, muscle force generation, and mitochondrial localization. The absence of desmin, the predominant IF in striated muscle, or keratin 19 (K19) results in the accumulation of mitochondria under the sarcolemmal membrane in both cardiac and striated muscle. However, the loss of both desmin and K19 does not result in subsarcolemmal accumulation of mitochondria. We hypothesize that the abnormal accumulation of subsarcolemmal mitochondria membrane is due to a defect in mitochondrial trafficking, normally regulated by MT and associated motor proteins. Immunofluorescence and expression of a YFP-mitochondrial construct in wild type (WT) and K19 knockout (K19 KO) Flexor digitorum brevis (FDB) fibers indicate that not only do mitochondria accumulate under the membrane, but that their morphology is altered in areas where MT are disrupted. We characterized MT organization and composition in FDB fibers from wild type (WT), desmin knockout (desmin KO), K19 KO, and desmin/K19 double knockout (DKO) mice. In WT mice, sarcolemmal MT are highly ordered, comprised of stable tubulin, and colocalize with dystrophin. Desmin KO muscle shows disruption of the sarcolemmal MT network, characterized by a decrease in tubulin overlying the Z-disk and bundles comprised of stable MT. The DKO results in highly disordered sarcolemmal MT comprised of unbundled, stable tubulin. In contrast, the K19 KO has a moderate disorganization of the sarcolemmal MT network, paired with a large decrease in the number of stable sarcolemmal microtubules. By depolymerizing the MT network, we find that the MT in desmin KO fibers collapses rapidly, leading to an accumulation of tubulin near the A-I junction of the contractile apparatus. Immunofluorescence studies of kinesin 3 show abnormal accumulation near areas of disrupted microtubules in desmin KO and K19 KO fibers. Finally, expression of the YFP-mitochondrial construct into WT FDB fibers followed by fluorescence recovery after photobleaching (FRAP) identifies two populations of mitochondria: stable, immobile mitochondria localized near the myofibrils, and mobile mitochondria under the sarcolemma. We conclude that the loss desmin, K19, or both proteins from skeletal muscle disrupts the normal organization, composition, and stability of the subsarcolemmal MT network. We tentatively conclude that the motile mitochondria found just under the sarcolemma move through an interaction between kinesin and the MT network, and that disrupting the sarcolemmal MT network can result in

abnormal subsarcolemmal accumulation of mitochondria and altered mitochondrial morphology. Future experiments will examine the mobility of the mitochondria in KO FDB muscle fibers, and how the individual components of the intermediate filament network, such as desmin and K19, are involved in cytoskeletal organization and organelle trafficking.
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18. A PROTECTIVE ROLE FOR RNASE-L IN COLITIS AND COLITIS-ASSOCIATED CANCER

Tiha M Long

Poster Presentation; Room 349; Basic Science 4

Colorectal cancer (CRC) represents the third most common cancer diagnosis in the United States in men and women and the second most common cause of cancer deaths in men and women combined. Furthermore, CRC diagnosis in individuals under 50 years of age has increased since 1998. An important risk factor for CRC is chronic inflammatory bowel disease (IBD), an immune disease of the gastrointestinal (GI) tract which is classified as Crohn s disease or ulcerative colitis (UC). UC patients have a 10-fold greater risk of developing CRC and exemplify the link between inflammation and cancer. To develop therapeutic strategies for Colitis-associated cancer (CAC), it is essential to determine the molecular mechanisms that regulate GI inflammation and the proliferative response of intestinal epithelial cells (IECs). Towards this goal my work focuses on RNase-L, an established component of the innate immune response that functions as a tumor suppressor in part by inhibiting cell proliferation and by regulating the induction of proinflammatory cytokines. We hypothesize that RNase-L confers protection from UC and CAC through the regulation of inflammatory mediators and through the maintenance of IEC homeostasis. Consistent with this prediction, our data demonstrated that colitis severity was increased in RNase-L-deficient mice in a model of UC. We also found that cytokine induction was altered in Rnasel-/- as compared to Rnasel+/+mice in this model. Furthermore, Rnasel-/- mice display increased GI tract permeability. Currently, we are using a murine model of CAC to determine if colorectal neoplasia is increased in Rnasel-/- as compared to Rnasel+/+mice; preliminary data indicate that the number of tumors is increased in Rnasel-/- mice. This study will provide information on the role and mechanism of action of RNase-L in UC and CAC and will serve as a foundation for the development of RNase-L-targeted therapies.

19. DEVELOPED OF THE CHARMM POLARIZABLE FORCE FIELD FOR PROTEINS BASED ON DRUDE OSCILLATORS

Pedro E.M. Lopes, Alexander D. MacKerell Jr.

Poster Presentation; Room 349; Basic Science 4

The newly developed polarizable CHARMM force field for proteins, based on the classical Drude oscillator, is presented. The steps undertaken over the years to develop the force field: (1) determination of bonded and non-bonded parameters of the small molecules making the building blocks of the larger biomolecules; (2) determination of the crucial electrostatic parameters and (3) adjustments needed to describe properties of larger polypeptides are described. Application of the force field to the simulation of small peptides and larger proteins in aqueous solution and solid phase is also presented. Structural and

dynamical properties are compared with available experimental data.

20. FLIM OF PHYSIOLOGICAL FREE CU(II) IN LIVING CELLS WITH A PROTEIN BASED BIOSENSOR

Bryan McCranor, Hui-Hui Zeng, Henryk Szmacinski, Carol A. Fierke, Joseph R. Lakowicz, and Richard B. Thompson

Poster Presentation; Room 349; Basic Science 4

Recently, the biology of copper ion has excited substantial interest not only in the biomedical field but in oceanography and the environment as well. Rae, et al., famously predicted (Science 284, 805 (1999)) that ordinarily no free copper ion would be found in resting cells based on the femtomolar affinity of a yeast copper chaperone. We have developed a biosensor that employs variants of human carbonic anhydrase II (CA II) with very high affinity and selectivity to quantitate free Cu(II) by changes in fluorescence lifetime. Using a membrane-penetrant CA II variant with much higher selectivity for Cu(II) over Zn (II) than wild type, we measured free Cu(II) levels by confocal fluorescence lifetime imaging microscopy in resting PC-12 cells in vitro. We found that the indicator system could be successfully calibrated in the cell, and that the resting level was very low, near the detection limit of ~10 femtomolar. The implications of the findings are discussed. The authors thank NIH for support (RO1-EB003924-05 [RBT, CAF, HHZ, and BJM] and RC1-GM091081-01 [JRL, HS, and RBT]) and ISS (Champaign, IL) for the use of the Alba confocal FLIM.

21. INTEGRATING A HETEROLOGOUS ANTIGEN INTO THE CHROMOSOME OF SHIGELLA USING THE TN7 TRANSPOSON SYSTEM

Carolyn Morris and Eileen Barry

Poster Presentation; Room 349; Basic Science 4

Shigella are Gram negative bacteria that cause diarrheal disease in humans. Shigellosis is most common in children and in developing countries where it is estimated that 600,000 children under age 5 die each year. Investigators at the Center for Vaccine Development (CVD) have created a live attenuated vaccine strain of Shigella flexneri 2a called CVD 1208S. In clinical trials, CVD 1208S was well tolerated and induced anti-LPS IgG and IgA, which are correlated with protective immunity. The goal of this project was to express the B subunit of the heat labile toxin (LTB) from Enterotoxigenic E. coli (ETEC) on the chromosome of CVD 1208S. Like Shigella, ETEC causes diarrhea in children in the developing world and is the predominant causative agent of travelers' diarrhea. Integration of LTB in the chromosome of CVD 1208S could potentially yield a multivalent vaccine that would offer protection against both Shigella and ETEC. We investigated the use of the Tn7 transposon system developed by McKenzie and Craig (BMC Microbiology, 2006) to integrate LTB into the Shigella chromosome. We hypothesized that the Tn7 system previously developed in E. coli could also be used in Shigella. Here we show that the Tn7 transposon system was able to specifically insert a heterologous gene into the chromosome of multiple Shigella species as confirmed by sequencing. We also show that CVD 1208S expressing LTB from a plasmid replicated similarly to parental CVD 1208S in HeLa cells and that bacterial gene expression could be measured from intracellular bacteria.

22. MD SIMULATION OF THE EFFECT OF OXIDIZED METHIONINE ON THE BINDING OF DNA AND CBFβ TO THE RUNT DOMAIN OF CBFα

Muhammad S. Noon, Maria Mochin-Peters, Antonino Passaniti and Alexander D. MacKerell, Jr.

Poster Presentation; Room 349; Basic Science 4

Runt domain (RD) is DNA-binding domain of p53 type transcription factors known as core binding factor α (CBFα) subunits. Three related genes Runx1, Runx2 and Runx3 are involved in the expression of CBFαs. Runx genes are important for several developmental processes; Runx1 plays role in haematopoiesis, Runx2 is important for skeletal development (osteogenesis) and Runx3 is essential in the development of gastric epithelium. Mutation of Runx1 is linked with human leukemias, whereas mutation of Runx3 leads to development and progression of gastric cancers and testicular yolk sac tumor. Runx2 gene mutation is associated with inherited human skeletal disorder known as cleidocranial dysplasia. Biological activity of some proteins is known to be sensitive to oxidative modifications caused by variety of oxidants. Methionine, and Cysteine, residues of proteins are particularly sensitive to oxidation by reactive oxygen species (ROS). Methionine residues may be oxidized to sulfoxide form (reversible) and to sulfone form (permanent change). Here, we carry out molecular dynamic (MD) simulation studies of RD, which is the DNA-binding region of Runx genes, to see the effect of oxidized (sulfoxide form) methionine-106 of RD on its interactions with its binding partners. Methionine-106 is located at the interface with a related protein, known as core binding factor β (CBFβ), which binds to the RD to enhance RD-DNA interaction. The simulation studies indicate that oxidation of methionine-106 leads to improved binding of RD with CBFβ and DNA as dimers and in the DNA-RD-CBFβ ternary complex.

23. INCIDENCE AND RISK FACTORS OF TOTAL KNEE ARTHROPLASTY (TKA) IN OLDER ADULTS WITH END-STAGE OSTEOARTHRITIS

Shannon O'Connor, Marc Hochberg

Poster Presentation; Room 349; Basic Science 4

While many older adults with end-stage knee osteoarthritis (OA) may benefit from total knee arthroplasty (TKA), only a small fraction undergo surgical treatment. The present study analyzed data from the Baltimore Clinical Centers of the Osteoarthritis Initiative (OAI), a multi-center, longitudinal, observational cohort study of older adults aged 45-79 years conducted between 2003 and 2009. This study examined the relationship of baseline parameters that may be predictive of TKA, including cohort, gender, race, age, previous knee injury, previous knee surgery, hand OA, and obesity. Out of 1,317 participants enrolled between 2003 and 2004, 60.7% were women and 51.1% were white. Participant cohort distribution was 65% incidence and 35% progression. A total of 75 participants underwent total knee arthroplasty over 4 years of follow-up; of these, 71 had unilateral TKA and 4 had bilateral TKA. The cumulative incidence rate over 4 years of follow-up was 4.18%.

24. A ROLE FOR STAT1 IN THE CONTROL OF SARS-COV PATHOGENESIS

Carly Page, William Funkhouser, Mark Heise, Ralph S. Baric and Matthew B. Frieman.

Poster Presentation; Room 349; Basic Science 4

The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) often caused

severe end-stage lung disease, organizing phase diffuse alveolar damage and death, especially in the elderly after infection. The virus-host interactions that govern development of these acute end stage lung diseases are unknown. To address this question, we evaluated the role of innate immune signaling in protection from a recombinant mouse adapted SARS-CoV, designated rMA15. rMA15 infection of type I, type II or type III interferon knockout mice resulted in minimal clinical disease outcomes, including transient weight loss, denuding bronchiolitis and alveolar inflammation and recovery, identical to that seen in infection of wildtype mice. This suggests that type I, II and III interferon receptors play minor roles in regulating SARS-CoV pathogenesis in mouse models. In contrast, infection of STAT1-/- mice resulted in severe disease, high virus titer, extensive pulmonary lesions and 100% mortality. These findings demonstrated that SARS-CoV pathogenesis is regulated by a STAT1 dependent but type I, II and III interferon receptor independent, mechanism. In rMA15 infected STAT1-/- mice we find a significant accumulation of alternatively activated macrophages (AAMs) suggesting a role for STAT1 either in the induction, proliferation or activation of AAMs. We are currently investigating the role of AAMs in SARS-CoV mediated severe lung disease.

25. ANTITUMOR EFFECTS OF ANTI-CD137 MAB ARE INDEPENDENT OF FC: ACTIVATING FCγ RECEPTOR INTERACTION

Michelle Sallin, Wei Lin, Andrei I. Chapoval, Dan H. Schulze, and Scott E. Strome

Poster Presentation; Room 349; Basic Science 5

In various preclinical models, agonist antibodies directed against CD137 (4-1BB) enhance the antitumor immunity. However, the contribution of the Fc:Fc receptor interactions to the process of anti-CD137 mediated tumor rejection is unknown. In this study, we provide early evidence that treatment of tumor bearing mice lacking all activating Fcγ receptors (Fcγ chain deficient mice) with the anti-CD137 mAb, 4-1BB2A, improves the survival compared to wild type mice. When Fcγ chain deficient mice and wild type are challenged with EL4E7 tumor cells, tumors were allowed to grow to an approximate size of 10mm by day 7 or 12mm by day 10. When the mice are treated with anti-CD137 mAb, the Fcγ chain deficient mice had increased survival compared to wild type mice even bearing ten day established tumors. Similarly, improved survival is seen in FcγRIII deficient mice bearing ten day established tumors after treatment with anti-CD137 mAb. While still preliminary, our observations suggest that strategies to modify Fc:FcR interactions may improve the therapeutic efficacy of anti-CD137 based cancer immunotherapy.

26. FOXO1 NUCLEAR-CYTOPLASMIC MOVEMENT IN LIVING SKELETAL MUSCLE

Tova Schachter & Martin F. Schneider

Poster Presentation; Room 349; Basic Science 5

The transcription factor Foxo1 is integral to the regulation of expression of proteins which promote muscle atrophy. Phosphorylation of Foxo1 causes its translocation to the cytoplasm and thus prevents Foxo1-DNA binding and consequent transcription of genes that cause muscle atrophy and cell death. Thus, phosphorylation of Foxo1 leads to cell survival and muscle hypertrophy. Maintenance of Foxo1 phosphorylation and its resulting cytoplasmic retention could be used to suppress muscle atrophy and thereby shift the atrophy/hypertrophy balance in favor of hypertrophy. This in turn may be utilized to develop therapeutic avenues in treatment of muscle wasting as seen in patients with denervation, age-

related muscle wasting, and AIDS. Akt and serum- and glucocorticoid-inducible kinase (SGK) are important regulators of the phosphorylation status of Foxo1. These pathways have been well characterized and the effects on Foxo1 localization have been reported. However, the mechanisms which regulate nuclear influx and nuclear efflux have not been separately evaluated. Here, we determine the effects of Akt kinase activity specifically on nuclear influx of Foxo1. To accomplish this goal, we quantified nuclear and cytoplasmic levels of adenovirally expressed Foxo1-GFP in cultured flexor digitorum brevis. The nuclear influx during treatment with kinase inhibitor Akt IV alone and in combination with the nuclear efflux inhibitor lemptomycin B provides insight into the activity of Akt as a Foxo1 kinase. Surprisingly, Akt inhibition reveals Akt to have little effect on the rate of nuclear influx of Foxo1. To evaluate translocation of endogenous Foxo1 in a similar manner immunocytochemistry and western blotting techniques were used. These results indicate that Akt either phosphorylates Foxo1 primarily in the nucleus and not in the cytoplasm, or that there is a mechanism for cytoplasmic phosphorylation of Foxo1 other than via Akt. Supported by NIH-NIAMS Grants R01-AR056477 and T32-AR007592.

27. T CELL EXPRESSION OF NOX2 REGULATES T HELPER DIFFERENTIATION

Kristen Shatynski

Oral Presentation; Room 351; Basic Science 2

Absence of phagocyte NADPH oxidase (NOX2) activity causes chronic granulomatous disease (CGD), a primary immunodeficiency characterized by recurrent bacterial infections. In seeming contradiction, immune responses to agents such as H. pylori and influenza imply an altered or augmented adaptive immune response in CGD. We have shown NOX2 expression in T cells, and oxidase deficient T cells demonstrate Th1 skewed cytokine secretion upon in vitro stimulation. The goal of this study was to determine if these differences are T cell inherent, or if other cells promote T helper polarization. Activation of purified naive CD4+ T cells from NOX2-deficient mice led to augmented IFN-γ --and diminished IL-4 production, consistent with a Th1 skewing of naïve T cells. This was supported by enhanced expression of the transcription factor Tbet and lower levels of GATA-3. Similar to NOX2-deficient cells, Th2-biased BALB/c T cells treated with the antioxidant N-acetyl cysteine produced increased IFN-γ and decreased IL-4 as compared to untreated controls. Inhibition of TCR-induced STAT5 phosphorylation was identified as a potential mechanism for skewed T helper differentiation. Stimulation of TCR-transgenic, OT-II T cells in vitro with NOX2-deficient splenic APC produced similar amounts of IL-4, IL-17, and IFN-γ as compared to those incubated with wild type APC, supporting the conclusion that NOX2 can regulate the adaptive immune response in a T cell inherent fashion.

28. CONFORMATIONAL SAMPLING OF TELITHROMYCIN/CETHROMYCIN AND DESMETHYL ANALOGS

Meagan C. Small, Rodrigo B. Andrade, and Alexander D. MacKerell, Jr

Poster Presentation; Room 349; Basic Science 5

Overcoming microbial resistance presents a major challenge in the development of new antibiotics. Approximately 50% of all antibiotics target the peptidyl transfer center (PTC) of the bacterial ribosome, inhibiting protein synthesis and thereby disrupting cellular growth. A recent class of 3rd generation macrolides, the ketolides, has been demonstrated to be effective against bacterial strains with macrolide-lincosamide-streptogramin B (MLSB)

resistance. This activity is due in part to the replacement of a C-3 cladinose sugar with a carbonyl, which bypasses a gene-encoded macrolide efflux mechanism (mef) and addition of an alkyl-aryl chain to a C-11,12 cyclic carbamate, which increases ligand affinity via interaction with a second site. However, ketolides are still susceptible to resistance via mutation mechanisms, the most common of which is A2098G (E.coli numbering). Crystallographic studies show that this is due to the presence of a C-4 methyl group that sterically clashes with G2098. To probe the role of the C-4 methyl group as well as those at C-8 and C-10 for structural simplification purposes, 20 nanosecond molecular dynamics simulations in explicit solvent were performed on telithromycin and cethromycin and their desmethyl analogs. Analysis of selected distances distributions revealed that the conformations sampled by the parent compounds and their desmethyl derivatives are significantly different. The probability maxima of the distances for both telithromycin and cethromycin overlap well with crystal structure distances. Surprisingly, the largest difference is observed for the least chemically modified analog in both ketolides studied, C-4 desmethyl(ceth/teli)thromycin. In addition, for both ketolides and analogs, desmethylation drastically changes the conformation of the macrocyclic ring, with lesser impact on the pyranose to alkyl-aryl arm distance.

29. TARGETING ZYMOGEN ACTIVATION TO CONTROL MATRIPTASE-PROSTASIN PROTEOLYTIC CASCADE

Zhenghong Xu, Ya-Wen Chen, Aruna Battu, Paul Wilder, David Weber, Wenbo Yu, Alexander D. MacKerell, Li-Mei Chen, Karl X. Chai, Michael D. Johnson, and Chen-Yong Lin

Poster Presentation; Room 349; Basic Science 5

Matriptase, a type II transmembrane serine protease, has been implicated in human diseases, including cancers, skin disorders, osteoarthritis and arteriosclerosis, and might be a potential drug target. Although several synthetic inhibitors targeting proteolytic activity of matriptase have been developed, these catalytic inhibitors may not be effective for the control of matriptase function due to the unusually short life span of active matriptase, a consequence of the rapid inhibition of active matriptase by its endogenous inhibitors. In the present study, a novel class of matriptase inhibitor targeting zymogen activation has been developed by a combination of the screening of compound library using a cell-based, ELISA-like matriptase activation assay and a computer-aided search of commercially available analogs of a selected compound. Four structurally related compounds are identified that can inhibit matriptase activation with IC50 at low μM in both intact-cells and a cell-free system, suggesting that these inhibitors target the matriptase autoactivation machinery rather than intracellular signaling pathways involved in matriptase activation. These activation inhibitors can also inhibit prostasin activation, a downstream event that occurs in lockstep with matriptase activation. In contrast, the matriptase catalytic inhibitor CVS-3983 at a concentration 300-fold higher than its Ki fails to inhibit the activation of both proteases. Our results suggest that inhibiting matriptase activation rather than matriptase proteolytic activity is an efficient way to control matriptase function. This study further reveals a novel strategy for controlling the matriptase-prostasin proteolytic cascade.

30. PHARMACOPOEIAL QUALITY OF SULFAMETHOXAZOLE/TRIMETHOPRIM DRUGS SUPPLIED BY PHARMACIES IN SIX COUNTRIES

Ting Wang, Neha Sheth, Maria Eng, James Polli, Carsen Holt, Stephen W. Hoag

Poster Presentation; Room 349; Basic Science 5

Purpose: To investigate the quality of antimicrobial drugs Sulfamethoxazole/Trimethoprim tablets obtained from six countries in Asia, Africa and South America, in terms of meeting United States Pharmacopieal requirements of content of active pharmaceutical gradients(APIs), the content uniformity of APIs and the tablet breaking force.
Methods: We randomly collected thirty samples of Sulfamethoxazole/Trimethoprim drugs from thirty pharmacies in seven areas of six countries in Asia, Africa and South America. Sulfamethoxazole and Trimethoprim standards were purchased from United States Pharmacopeia. We analyzed the medicines for drug content by chromatographic methods as U.S. Pharmacopeia (U.S.P.) described in the Sulfamethoxazole and Trimethoprim tablet monograph. The content uniformity and crushing strength of all formulations were also determined using the methods as U.S.P. stated. We compared the amount of active drug present in the samples and content uniformity with the limits specified in the U.S.P.. Near Infrared Spectroscopy was also applied for the tablets analyzed.
Results: The assay results showed that the percentage of Sulfamethoxazole in the samples ranging from 98.5% to 107.2% of the labeled content and that of Trimethoprim from 93.6% to 105.0%. Therefore all samples passed the USP 32 requirements for drug content (93-107% of the labeled content). Five formulations of sulfamethoxazole/trimethoprim tablet with relative standard deviation (RSD) of 2.21, 2.25, 2.38, 3.23 and 5.97% failed to meet the USP 32 requirement (RSD of the drug substance of the drug in each tablet is no more than 2%). The breaking force of the 30 samples range from 4.8 kP to 19.3 kP. A few tablets from two samples with average breaking forces of 4.8kP and 11kP were broken during handling before assay. Coefficient of variation of the tablet breaking force went from 1.7% up to 41%.
Conclusion: The results indicate that none of the samples marked in the six countries were substandard with respect to U.S.P. content of APIs standards. However, five out of thirty samples (16.7%) did not meet the U.S.P. content uniformity requirements although they are close to pharmacopoeial limit. And the tablet breaking force spread out for a wide range, some of them have relative wide variation (standard deviation). The comprehensive qualities of some samples were not as satisfied as U.S.P. required.

31. A NOVEL THERAPEUTIC TARGET IN A MODEL OF ENCEPHALOPATHY OF PREMATURITY IN RATS

Cigdem Tosun, Michael T. Koltz, David Kurland, Volodymyr Gerzanich, J. Marc Simard

Poster Presentation; Room 349; Basic Science 5

Encephalopathy of prematurity (EP) is common in preterm, low-birth weight infants who require postnatal mechanical ventilation. The worst forms of EP include choroid plexus and germinal matrix hemorrhages, which may extend intraventricularly. Survivors exhibit life-long cognitive, behavioral and motor abnormalities. Available preclinical models do not fully recapitulate the salient features of the human disease. Here, we evaluated a rat model that features tandem insults of prenatal transient ischemia plus postnatal transient venous hypertension.
We simulate the human condition of EP in our novel rodent model by inducing intrauterine ischemia (IUI) on E19 by clamping the uterine vasculature for twenty

minutes followed by reperfusion. Natural birth occurred on E22. Six hours after birth, pups were subjected to an episode of venous hypertension (VH), induced by injecting 50% glycerol IP. The SUR1-regulated NCCa-ATP channel, which requires hypoxic/ischemic insult for de novo expression and channel opening, has been previously implicated as a key player in mediating endothelial cell dysfunction in the germinal matrix of preterm infants. Blocking the channel by the potent and safe sulfonylurea, glibenclamide (glyburide), has been shown to attenuate such dysfunction in rodent models of stroke and spinal cord injury. Here, we show that IUI complicated by venous hypertension prominently upregulates SUR1, predisposing periventricular tissue to infarction, hemorrhage and subsequent leukomalacia. Additionally, treatment with glibenclamide significantly reduces mortality and the acute histopathological manifestations likely associated with the opening of SUR1-regulated channel. Rat pups that survived the model also exhibited significant reduction in brain and body weight that was recovered in the treatment group. In order to determine the long-term benefits of glibenclamide, we assessed rats to well-studied vestibulomotor, behavioral and learning paradigms until P52. Compared to controls, pups subjected to IUI+VH exhibited vestibulomotor abnormalities, and significant deficits in rapid spatial learning when tested in the Morris water maze. Importantly, these deficits observed in our model are significantly attenuated by treating the dam with low-dose glibenclamide prior to birth.
In conclusion, the present study with IUI plus postnatal VH recapitulates many features of the hemorrhagic forms of EP found in humans, suggesting that these insults in tandem may play important roles in pathogenesis. Administration of glibenclamide significantly improves the observed morbidities and preserves long-term cognition in a clinically relevant rodent model of EP.

32. DEVELOPMENT OF PUTATIVE LINEAGES OF NURSE SHARK PLASMA CELLS

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

Poster Presentation; Room 349; Basic Science 6

IgM was the first immunoglobulin isotype to arise in evolution. Cartilaginous fish, including sharks, skates and rays, are the oldest animals with Ig isotypes. They express IgM in two forms, the typical pentameric (19S) form, which is attached to J-chain, and a monomeric (7S) form devoid of J- chain. Preliminary evidence suggests that shark 19S IgM consists of low affinity antibodies. In contrast, 7S monomers are able to develop a specific, high affinity response to an antigenic challenge. In neonatal nurse sharks the great majority of plasma cells express J-chain and produce pentameric IgM. Neonatal nurse sharks also express a germline-joined isotype, IgM1gj, at high levels. One of two scenarios likely occurs during plasma cell development: 1) 19S producers "switch" to become 7S producers by shutting down J-chain expression after activation by antigen and T cells, or 2) The two populations develop from completely separate lineages, with the 7S, J-chain-negative lineage arising later in development than the 19S lineage. Additionally, there may be two lineages of 19S-secretory cells, one arising early in development and the other developing throughout the life of the animal. To examine these potential lineages in the nurse shark, we are looking at differences in developmental timing and gene expression bias in 19S- and 7S-producing cells, and differential expression of transcription factors. Preliminary results suggest that IgM1gj secreting cells appear first, followed by cells secreting early, rearranging 19S IgM and expression of a unique light-chain, sigma-prime (s'). Later, another wave of 19S-secreting cells and J-chain-negative 7S IgM secretors appear. Surprisingly, we have also found that the IgM1gj and 19S

IgM secreting cells differ from 7S secretors in that they do not express Blimp1, the "master regulator" of plasma cell development, whereas the presumably antigen-reactive, 7S-secreting cells express high levels of Blimp1.

33. Estradiol Alters Transcription Factor AP2-gamma Gene Expression and DNA Methylation

Jerry D Choate, Zachary A Kaminsky, Dubravka Jancic, James B Potash

Poster Presentation; Room 349; Basic Science 6

Post-partum depression is associated with the precipitous decrease of female hormones following parturition. A recent analysis by our group identified an enrichment of transcription factor AP2-gamma (TFap2c) target genes in a genome wide association study dataset of post-partum depression. Members of the AP2 family of transcription factors regulate expression of several genes and have been associated with psychiatric disorders in women. Given the presence of an estrogen response element in the promoter of TFap2c, fluctuations in TFap2c expression, and its target genes, during the postpartum period may manifest as changes in mood. We tested the hypothesis that TFap2c and a representative target gene Bcl6 corepressor (Bcor) expression would be modulated by estradiol and mediated by changes in TFap2c DNA methylation. To this end, H19-7 rat hippocampal neuronal cells were treated with estradiol for 24h and evaluated for TFap2c expression and DNA methylation status by quantitative real time PCR and sodium bisulfite pyrosequencing, respectively. DNA methylation of TFap2c was increased with estradiol. Also, TFap2c and Bcor mRNA levels were decreased in estradiol-treated cells, compared to controls. DNA methylation of CpGs assayed in the TFap2c 3'-UTR correlated negatively with expression of Bcor and is mediated by the expression of TFap2c. In addition, preliminary data from SH-SY5Y human neuroblastoma cells treated for 24h with estradiol shows increased methylation of CpGs in the TFap2c 3'-UTR. Elucidation of mechanisms underlying TFap2c expression and DNA methylation in relation to steroid hormones is ongoing and may provide biological basis for mood changes during hormone fluctuation.

34. Effects of menstrual cycle phase on task-directed attention during pain processing

Timothy J Meeker, Dieuwke S Veldhuijzen, Michael Keaser, Rao P Gullapalli and Joel D Greenspan

Poster Presentation; Room 349; Basic Science 6

Several studies report significant differences in pain sensitivity across the menstrual cycle in women. Differential activation in response to similarly rated painful heat stimuli were reported in the anterior cingulate cortex, precuneus and cerebellum when comparing menstrual and follicular phases. These areas have been implicated in the interaction of pain and cognitive load. It remains unknown if hormonal changes over the menstrual cycle modulate brain activation in response to task directed attention in the presence of painful stimuli. The present study explores the cerebral response to an attention-demanding task while painful electrocutaneous stimuli are applied across the menstrual cycle of healthy women. Twelve participants underwent four fMRI sessions during their menstrual, follicular, periovulatory and luteal phase. The cerebral response to nine task-stimulus pairs was determined. The task levels included a low-demand task, a high-demand task and a motor task. The stimulation levels included a no stimulus, a non-painful stimulus and a painful stimulus (about 60 on a 0-100 VAS). Within-subject analyses were performed on whole brain

activation using a 3-way RM-ANOVA. Painful electrical stimuli were perceived as similar in intensity over the menstrual cycle, as intended by design (RM-ANOVA on ranks: §2=5.979, p=0.113). Pain unpleasantness ratings varied across the cycle (F=3.252, p=0.031) with lower ratings during the follicular versus the luteal phase (p=0.041). Differences between follicular and menstrual phase (p=0.056) or periovulatory phase (p=0.85) were not significant. The RM-ANOVA (cycle phase x task x stimulus) revealed numerous regions of phase main effect and interactions of phase with stimulus and task level. The main effect of phase revealed 34 clusters at p<0.05. The interaction between cycle phase and stimulus revealed 24 clusters, while the interaction between cycle phase and task revealed 25 clusters at p< 0.05. This is the first study to explore the cerebral response to cognitive load and painful stimulation across the menstrual cycle. Many regions expected to be engaged by painful stimulation or by task demands showed significant variation in response across the menstrual cycle. The effect of cycle phase per se showed a consistent pattern across many brain regions, with higher responses during the menstrual phase vs. other phases.

35. Regulation of bone mineralization by Transglutaminase-mediated cross-linking in Zebrafish

Stephanie Deasey, Shaojun Du, Olga Grichenko, Maria Nurminskaya

Poster Presentation; Room 349; Basic Science 6

The protein cross-linking enzyme transglutaminases (TGs) play crucial roles in many biological processes and diseases. Previous in vitro studies have implicated a role for mammalian Transglutaminase 2 (TG2) and Factor XIII (FXIIIa) in promoting osteoblast differentiation and bone mineralization, however mouse models lacking either gene have no skeletal phenotype likely due to a compensation effect. Here we present the first in vivo evidence for the regulation of bone mineralization by transglutaminase-mediated cross-linking. We have employed zebrafish (Danio rerio) to study the role of TGs in bone formation. Based on analysis of genomic organization and phylogenetics, we report the identification of seven zebrafish TGs with high homology to only three mammalian enzymes -TG1, TG2 and FXIIIa. In addition, we identify distant homologues for TG5 and TG6, but not for TG3, TG7 and EB4.2. We also show that zebrafish TG genes have distinct temporal expression profiles in larvae and adult fish, with zFXIIIa and zTG1 expressed during tail regrowth, implicating their role in bone formation. Mineralization of the newly formed vertebrae depends on TG-mediated cross-linking and is significantly reduced in fish grown for 5 days in the presence of TG inhibitor KCC-009 added at 3-5 days post fertilization. This treatment reduces average vertebrae mineralization by 30%, with complete inhibition in some fish, and no effect on the overall growth and vertebrae number. This is the first in vivo demonstration of the crucial requirement for the TG-catalyzed cross-linking activity in bone mineralization.

36. Basal forebrain Kv2.2-GABAergic neurons are involved in promoting wakefulness in the mouse

Tracey O. Hermanstyne , Jessica A. Mong and Hiroaki Misonou

Poster Presentation; Room 349; Basic Science 6

Sleep is essential for proper functioning of the brain. Disorders in the sleep-wake cycle can produce deficits in both learning and motor behaviors. In addition, sleep disorders can diminish the overall quality of life and in extreme cases cause death. Furthermore, sleep disorders have been

associated with other health consequences such as depression, anxiety, diabetes and cardiovascular diseases. It has been estimated that nearly 70 million Americans suffer from disorders of the sleep-wake cycle leading to billions of dollars in medical expenditures and categorizing it as a major public health issue.

Studies have shown that the induction and maintenance of the sleep/wake cycle incorporates multiple brain regions and neurotransmitters that contribute its own unique involvement in sleep physiology. However, out of those various brain regions, the basal forebrain (BF) is known to play an important role in the modulation of both sleep and wake states.1,2 Recognized as a region of the brain with a heterogeneous neuronal population, early studies have revealed both cholinergic and GABAergic neurons are the major constituents of the neuronal population in this area.3,4 Previous studies using specific lesioning techniques have demonstrated that BF cholinergic neurons are involved in wakefulness and cortical activation.5 However, due to the heterogeneity of the BF GABAergic neurons and lack of a specific molecular tool to study them, the contribution of how the GABAergic population regulates the sleep-wake cycle has not been clearly defined.

We have recently reported that there is a subset of neurons in the BF of the rat and mouse that have high expression levels of a voltage-gated delayed rectifier potassium channel Kv2.2. More specifically, this novel expression of Kv2.2 is in two nuclei of the BF; the magnocellular preoptic area (MCPO) and the horizontal diagonal band of Broca (HDB) both of which have been implicated in sleep behavior.6 Immunohistochemical studies from GAD67-GFP knock-in mice sections revealed that Kv2.2 expression is selective to 60% of the multipolar, GABAergic neurons which have similar morphology to cortical projecting neurons in this area. As a result of this observation, we hypothesize that the Kv2.2-GABAergic neurons of the MCPO/HDB are a novel and major subpopulation of BF GABAergic neurons that are involved in the sleep-wake cycle.

To test the hypothesis, we analyzed and compared the sleep-wake behavior of the Kv2.2 knockout (KO) mice to their wild-type littermates. Our data revealed that Kv2.2 KO mice spend a greater percentage of time awake, particularly during their dark period, as compared to their wild-type littermates. In addition, Kv2.2 KO mice exhibit an increase in their average duration of wake bouts, a decrease in the total of transitions between vigilant states, and a rightward shift in the cumulative probability plot for the length of wake episodes. These findings indicate that Kv2.2-GABAergic neurons promote wakefulness and by removing Kv2.2 expression there was a resultant change in the overall sleep architecture. Furthermore, Kv2.2 KO mice show a decrease in their EEG delta power, which suggest changes in cortical activity and quality of slow wave sleep. Lastly, a neuronal activity assay have shown that Kv2.2-GABAergic neurons are more active during a consolidated wake period as compared to a period of sleep recovery. Guided by these findings, we believe that Kv2.2 can be used as a molecular tool to target this major subpopulation of BF GABAergic neurons, to help identify the functional role of these neurons and can possible lead to the development of therapeutic drugs.

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37. HER2 SIGNALING REGULATES FN14 RECEPTOR EXPRESSION IN BREAST CANCER CELLS

Kaushal V. Asrani and Jeffrey A. Winkles

Poster,Oral Presentation; Room 349; Basic Science 6

Human epidermal growth factor receptor (HER)-2 overexpression occurs in ~25% of all breast cancers and these cancers have a very poor prognosis. HER2 requires HER3 to mediate cellular transformation and the HER2-HER3 heterodimer functions as a powerful oncogenic unit. Heregulin (HRG)-1 is a natural ligand for HER3 and elicits the formation of potent HER2-HER3 heterodimers. HRG is expressed in ~30% of breast tumors and is also a risk factor for invasive breast cancer. Fibroblast growth factor-inducible 14 (Fn14), a member of the TNF receptor superfamily, is overexpressed in breast tumors, where high expression levels strongly correlate with both the invasive HER2+/ER- intrinsic subtype and indicators of poor prognosis. Hence, we investigated whether HER2 activation in breast cancer cells could directly induce Fn14 gene expression. We found that transient or stable transfection of MCF-7 cells with HER2 increased Fn14 protein expression. Breast tumor tissue from HER2-transgenic mice showed increased expression of Fn14, over normal mammary gland. Also, HRG-1 induced Fn14 protein expression in a dose- and time-dependent manner in MCF-7 cells. Both the HER2 and HRG-1-induced increase in Fn14 expression in MCF-7 cells could be blocked by treatment with the HER1/2 tyrosine kinase inhibitor lapatinib. Also, siRNA knockdown of HER2 and/or HER3 decreased Fn14 expression in HER2-overexpressing cells, as did treatment with the MEK inhibitor U0126 and the PI3K inhibitor Wortmannin. Preliminary results indicate that Fn14 may play a role in regulating anchorage-independent growth in SKBR3 cells. Studies are in progress to elucidate the mechanisms involved in the increase in Fn14 expression and whether Fn14 contributes to HER2-triggered proliferation, migration and invasion. These studies should indicate whether Fn14 could be a new therapeutic target for HER2+ tumors with either intrinsic or acquired resistance to HER2-targeted drugs.

38. URINARY INCONTINENCE AND FALLS AMONG OLDER MEDICARE BENEFICIARIES

Sarah K. Dutcher, Ilene H. Zuckerman, Bruce Stuart, Gail B. Rattinger

Poster Presentation; Room 349; Informatics/Policy/Social Science 1

Objective: To examine the relationship between frequency of urinary incontinence (UI) and occurrence of falls in a nationally representative sample of older Medicare beneficiaries.
Methods: This cross-sectional study pooled 2003-2005 data from the Medicare Current Beneficiary Survey; individuals contributed up to three years of data. Individuals were included if they were e65 years old and community-dwelling. Information on UI symptom frequency and annual number of falls was obtained via self-report. Other characteristics included: demographics, comorbidities, medication use, functional status, and living situation attributes. Bivariate analyses and Ç2 tests assessed the unadjusted relationship between UI frequency and falls. A negative binomial regression model was used to quantify the adjusted relationship, with generalized estimating equations to account for within-respondent correlation.
Results: 26,641 person-years met the inclusion criteria, representing 16,011 unique beneficiaries. The mean age was 76.8 (±7.5) years and 56.7% were female. Almost 28% of beneficiaries reported involuntarily losing urine at least once in the previous year. Falling in the previous year was reported by 22.5%, of which 43% experienced recurrent falls. The proportion of those who reported falling was highest

among those with UI symptoms more than once per week (36%) and declined with decreasing UI frequency; only 19% reported falling among those with no UI ($p<0.0001$). Multivariate analyses demonstrated a 35% (95% CI 1.18-1.54) (UI donce per month) to 63% (95% CI 1.40-1.89) (UI twice per month) increased rate of falling annually, compared to no reported UI. Female sex, non-white race, and Hispanic ethnicity were associated with a lower rate of falling; age, certain comorbid conditions, and antidepressant or narcotic use demonstrated increased fall risk. Conclusions: An increased rate of falls is seen with higher UI symptom frequency among older Medicare beneficiaries. Decreasing UI through behavioral or environmental modifications, or pharmacotherapy are potential interventions through which the risk of falling may be lowered.

39. VICARIOUS TRAUMA SCALE: AN EXPLORATORY FACTOR ANALYSIS

Elizabeth Aparicio

Poster Presentation; Room 349; Informatics/Policy/Social Science 1

Vicarious trauma (VT) involves shifts in cognitive schemas following secondary exposure to traumatic material. The Vicarious Trauma Scale (VTS) is the only publicly available, brief measure designed to assess distress resulting from such exposure, and has great potential as a screening tool for VT in social work practice and research settings. The current study is the first examination of the factor structure of the VTS in a sample of licensed social workers (N=157) collected in a cross-sectional survey. Results of an exploratory factor analysis suggest VT is a multidimensional phenomenon and the VTS is a helpful screening tool of exposure to traumatic material or distressed clients and of the emotional and cognitive impact of such exposure. However, the VTS does not appear to measure shifts in social workers cognitive schemas in order to integrate the traumatic material, which is the final step in the development of VT. Implications for research and applications to social work practice are discussed.

40. THE CAMPUS HEALTH AND SAFETY SURVEY

Jayme Delano, Tammy Fish, Seokho Hong, Karen McNamara, Bethany Backes, Brenda Jorden, Mark Lardner

Poster Presentation; Room 349; Informatics/Policy/Social Science 1

The Campus Health and Safety Survey (CHASS) is an original anonymous online questionnaire exploring professional and graduate students' experiences with fear of crime and crime victimization at an urban university. Although a large body of literature exists on campus safety and victimization in the United States, few studies have examined these issues on a graduate level campus. Variables of interest include demographic and academic characteristics, behaviors, health, and past victimization. Results will provide preliminary information related to student crime victimization and fear of crime. Areas explored will include demographics, school affiliation, academic performance, substance use, social supports, use of campus resources, attitudes toward health, and past victimization. This poster will illustrate the methodological framework for this study that is anticipated to begin in late summer 2011.

41. THE EFFECTS OF EXERCISE-BASED REHABILITATION ON BALANCE AND GAIT FOR STROKE PATIENTS: A SYSTEMATIC REVIEW

Minjeong An

Poster Presentation; Room 349; Informatics/Policy/Social Science 1

This review evaluated the effects of balance and/or gait exercise interventions for stroke survivors and summarized the available evidence on these exercise interventions. A search for studies published between January 2001 and January 2010 was performed using the keywords: stroke, walking or balance, and physical activity or exercise. Seventeen randomized clinical trials were identified. The findings suggest that initiating early rehabilitation during acute to subacute stroke recovery can improve balance and walking capacity. The findings also demonstrate that at least 1 hour, 3 to 5 times per week, of balance training, and 30 minutes, 3 to 5 times per week, of gait-oriented exercise are effective to improve balance and walking. This review confirms that balance and walking capacity are improved with specific exercise modalities. A combination of balance, gait, and aerobic exercises would be ideal.

42. USING A RASCH ANALYSIS TO ASSESS RELIABILITY AND VALIDITY OF THE JOB ATTITUDE SCALE MEASURING JOB SATISFACTION IN NURSING ASSISTANTS IN NURSING HOMES AND ASSISTED LIVING FACILITIES

Kelly Flannery, RN, MS, PhD (c); Barbara Resnick, PhD, RN, CRNP, FAAN, FAANP

Poster Presentation; Room 349; Informatics/Policy/Social Science 1

Background: Given the rapidly increasing number of older adults, the need for nursing assistants (NAs) has multiplied. There is a need to establish and assure adequate job satisfaction as one way to keep NAs in the profession and ideally remaining within the setting in which they work.
Purpose: Our purpose is to provide continued support for the reliability and validity of a common measure to assess job satisfaction in NAs that work in nursing homes (NHs), the job attitude scale (JAS). We also aim to provide new evidence of reliability and validity of the JAS when used with NAs working in assisted living facilities (ALs).
Methods: We used a combination of traditional test theory (Cronbach's alpha and known group validity testing by independent T test) and Rasch analysis to test the reliability and validity of the JAS.
Results: Reliability proved to be sufficient as evidence of person separation indexes (range 1.46-1.65), item separation reliability (range .98-.99) and Cronbach's alphas (range .71-.73). Most items in the model proved to be valid as evidence by sufficient infit (range .77-1.71) and outfit statistics (range .72-2.12). Generalizability proved to be sufficient as evidence by an item separation index of 4.31-12.81. Validity was also proven when ALs JAS scores were significantly higher than NHs JAS scores (t=-3.313, p.001).
Conclusion: To our knowledge the psychometric statistics provided in this article make the JAS the most psychometrically tested instrument to assess job satisfaction with NAs. The Rasch analysis showed the JAS is a valid and reliable tool to measure job satisfaction in NAs in NHs. We also add new evidence of reliability and validity of the JAS in NAs in ALs.

43. BODY MASS INDEX AND BONE MINERAL DENSITY IN ADULTS OVER 50: RESULTS FROM NHANES 2005-2008

Jennifer Lloyd, Dawn Alley, William Hawkes, Shari Waldstein, Marc Hochberg, Denise Orwig

Poster Presentation; Room 349; Informatics/Policy/Social Science 1

Although previous studies have reported a positive relationship between body mass index (BMI) and bone mineral density (BMD), this relationship hasn t been examined in more recent cohorts. Using data from the National Health and Nutrition Examination Survey (2005-2008), we examined the association between BMI and low BMD and osteoporosis (defined as >1 SD and >2.5 SD below young, sex-specific mean, respectively). There were 3,801 adults >50 years (mean 63 years), predominately female (51.75%), white (83.52%), and overweight or obese (72%), with a mean femoral neck BMD of 0.78 gm/cm2 (SD=0.14). Both overweight and obese persons had significantly lower odds of low BMD (OR=0.33, CI: 0.28-0.39; and OR=0.14, 95% CI: 0.12, 0.17, respectively) and osteoporosis (OR=0.19, 95% CI: 0.08, 0.44; and OR=0.21, 95% CI: 0.08, 0.59, respectively). Gender and race were not significant effect modifiers. Results demonstrate the strong positive association between BMI and BMD, consistent with prior research.

44. A NOVEL APPROACH TO IMPROVE HEALTH STATUS MEASUREMENT IN OBSERVATIONAL CLAIMS-BASED STUDIES OF CANCER TREATMENT AND OUTCOMES

Amy J. Davidoff, Ilene H. Zuckerman, Naimish Pandya, Franklin Hendrick, Xuehua Ke, Arti Hurria, Stuart Lichtman, Arif Hussain, Jonathan Weiner, Martin J. Edelman

Poster Presentation; Room 349; Informatics/Policy/Social Science 2

Research Objective: Performance status (PS) is a measure of general functional status used by oncologists to select cancer treatment modality and intensity, and is an independent predictor of survival. Observational studies of treatments and outcomes using administrative data such as insurance claims represent a key component of comparative effectiveness research (CER) in cancer. One limitation of claims-based comorbidity measures is that they tend to capture the presence of conditions, but not the dimension of functional or PS. Hence, estimates from studies that rely on diagnosis based comorbidity measures are subject to potential indication bias. Our objective was to develop and validate a multivariate prediction model for PS, based on healthcare service use indicators developed from administrative claims, that could be used to improve covariate control in CER studies.
Study Design: We used the Medicare Current Beneficiary Survey (2001, 2003, 2005), a household survey of the Medicare beneficiary population that includes self-reported measures of functional status, strength, stamina, and exercise and is linked to Medicare insurance claims. A clinician panel guided development of an algorithm that linked the self reported measures to specific levels on the five-level Eastern Cooperative Oncology Group (ECOG) PS scale. We grouped further to create a dichotomous measure of good versus poor PS. Potential claims-based predictors were identified by clinicians based on an expected relationship to PS. Selected indicators were generated by modifying the Berenson-Eggers-Type-of-Service scheme for grouping Healthcare Common Procedure Codes. We used stepwise logistic regression to facilitate model selection. Optimal models were selected based on model fit, sensitivity, specificity, and predictive value. We conducted sensitivity analyses of models

with and without interactions. We used a split-half approach to test out-of-sample prediction, and examined model fit for a variety of subgroups.
Population studied: Medicare beneficiaries, N = 15,678.
Principal findings: Over one-tenth of beneficiaries met the definition for poor PS. Indicators associated with an increased probability of poor PS included having multiple evaluation and management physician visits, nursing home care, hospice, durable medical equipment such as wheelchairs and parenteral nutrition, while indicators of good PS included cancer and other disease screenings, immunizations, dermatology and chiropractic visits, electrocardiography and cardiac stress testing. Interactions between region and/or Medicaid enrolled with selected indicators were significant. The model without interactions yielded high sensitivity (0.80) and specificity (0.92); positive predictive value=55.2% and negative predictive value=97.4%, with c-statistic=0.92 and good model calibration. The model with interactions had similar characteristics but failed calibration tests. Adjustment of thresholds used to create dichotomous predictions altered the positive predictive value. Models performed equally well in the estimation and validation half, but did not perform well among non-elderly disabled beneficiaries.
Conclusion: Insurance claims based indicators, in combination with selected demographic characteristics, provide clinically meaningful predictors of PS. Model characteristics indicate good fit and predictive ability.
Implications for Policy, Delivery or Practice: The ability to assess PS from claims should significantly improve the validity of results from observational CER studies using data such as the Surveillance, Epidemiology and End Results registry linked to Medicare claims. Ongoing research is designed to validate use of predicted PS in CER studies of cancer treatment.

45. Vicarious Trauma in Social Workers: The Role of Trauma History, Social Support, and Years of Experience

Michalopoulos, Lynn & Aparicio, Elizabeth

Poster Presentation; Room 349; Informatics/Policy/Social Science 2

Vicarious trauma (VT), a disruption in schemas and worldview often accompanied by PTSD-like symptoms, occurs as a result of chronic secondary exposure to traumatic material. The aim of the current study was to examine the role of personal trauma history, social support, and experience level in the development of VT among licensed social workers in Maryland (N=160). Results indicated an increase in social support and in experience level of social workers predicted less severe VT. In addition, an interaction effect between trauma history and social support trending on significance indicated higher levels of social support may help protect those without a trauma history but not those with a trauma history against VT. Research and clinical implications are discussed.

46. Serious Mental Illness and Hospital Readmission in Older Diabetic Adults

Albrecht, J.S., Hirshon, J.M., MD, MPH, Goldberg, R., PhD, Day, H.R., MS, Morgan, D.J., MD, Comer, A.C., MPH, Harris, A.D., MD, MPH, Furuno, J.P., PhD

Poster Presentation; Room 349; Informatics/Policy/Social Science 2

Serious mental illness (SMI) has been associated with increased risk of hospital readmission in older adults with heart failure. However, to our knowledge, the impact of SMI on hospital readmission has not been assessed in diabetics. Patients with SMI have higher prevalence of diabetes but may receive lower quality of

diabetes care. Older adults with SMI could be especially vulnerable because they are at greater risk of both diabetes and hospital readmission. We hypothesized that among diabetics aged >65, SMI would be associated with increased risk of 30-day hospital readmission. We conducted a retrospective cohort study including all admissions to the University of Maryland Medical Center between February 1, 2005 and January 31, 2009 with diabetes (ICD-9 code 250.XX) included as a discharge diagnosis. Our primary exposure variable was a co-occurring diagnosis of SMI, defined by the presence of discharge diagnoses codes for schizophrenia, schizo-affective disorders, bipolar and manic disorders, major depressive disorder or other psychosis,. Our primary outcome was readmission to the index facility within 30 days of discharge. Generalized estimating equations were used to account for repeated outcomes. Among all adult admissions, 26,878 (16.5%) patients had diabetes and of these 8,992 (33%) were aged >65. Diabetic patients with SMI were not at increased risk [OR 1.00 (95%CI 0.79-1.26)] of 30 day hospital readmission compared to diabetic patients without SMI, controlling for age, length of stay, female sex and Charlson Comorbidity Index score. However, diabetic patients >65 had decreased odds [OR 0.78 (95%CI 0.71-0.85)] of 30-day hospital readmission compared to those <65, controlling for SMI, length of stay, female sex and Charlson Comorbidity Index score. Our study observed no association between SMI and 30-day hospital readmission in older diabetics. Interestingly, older adults with diabetes in our study were at a lower risk of 30-day hospital readmission. Better understanding of the impact of both SMI and diabetes on readmission in older adults may aid in allocation of limited case management resources.

47. EXPLORING THE ROLE OF INFORMAL SOCIAL NETWORKS IN THE DAILY LIVES OF LATINO IMMIGRANT DAY LABORERS

Lynn Murphy Michalopoulos, MA, LCSW, Adrianna Overdorff, BA, Dr. Nalini Negi, PhD, MSW

Poster Presentation; Room 349; Informatics/Policy/Social Science 2

Introduction:
Latino immigrant day laborers are a rapidly increasing population in the United States. These laborers, who are predominantly undocumented, face dangerous working conditions, exploitation and discrimination. These difficult life and work conditions are exacerbated by this population's lack of access to health care and social services. In the presence of significant barriers to care, social network theory postulates that marginalized populations, such as Latino immigrant day laborers, often seek the support of informal networks to meet their needs. As this is an under-studied area of research, this presentation aims to qualitatively understand the role of social networks in facilitating the well-being of Latino day laborers (LDLs).

Method:
This ethnographic study used participant observation, informal interviews, and focus groups to explore how LDLs build social networks as newly arrived Spanish monolingual immigrants to the United States and the importance of these networks in their daily lives. Over a two-year period, multiple researchers observed and recorded verbatim accounts and interactions and conducted informal interviews at three informal day labor sites in Austin, Texas with 150 LDLs. Two focus groups were also facilitated to further contextualize the meaning of well-being in LDLs' lives. Thematic analysis was utilized to examine ethnographic and focus group data to elucidate common themes across participants.

Results:
Findings indicated that LDLs face significant barriers to formal social services and often

rely on their social networks to enhance their well-being and promote their economic survival in the United States. Peer, family and church networks were identified as being critical for practical, financial and social support. Furthermore, LDLs revealed that social networks provided them with emotional nurturance and a sense of community.

Discussion:
This study provides an initial understanding of the complex social networks Latino migrants in the United States must develop in order to ensure their well-being and health. Public health and social service providers must be prepared to the meet the needs of this socially vulnerable and under-served population. Findings can be used to inform the development of strengths based and culturally responsive services and programs with this population.

48. Education and Functional Recovery from a Hip Fracture

Nancy Chiles, Ann Gruber-Baldini, Denise Orwig, Jay Magaziner

Poster Presentation; Room 349;
Informatics/Policy/Social Science 2

Cognitive ability impacts recovery from hip fracture and education positively correlates with cognition, but the details of the relationship between education, cognition, and hip fracture recovery remains unclear. This study hypothesizes that education would be an independent predictor of post-fracture cognition and improved functional recovery post hip fracture. 163 patients from an ongoing study (Baltimore Hip Studies, BHS-7, 75 male and 79 female), recruited from 8 Baltimore-area hospitals with a non-pathologic hip fracture. Baseline Modified Mini-Mental State (3MS) scores were used to determine cognitive impairment. Functional Recovery was measured by Lower Extremity Physical Activities of Daily Living (LPADLs) at 2 months post-fracture. At baseline, 77.3% where not cognitively impaired (3MS>=78), while 12.9% were moderately impaired (3MS 66-77) and 11.7% were severely cognitively impaired (3ms<66). Compared to non-cognitively impaired, severely cognitively impaired patients were older (M=85.8+/-10.1 vs. M=81.0+/-7.8), less educated (M=11.3+/-2.9 vs. 13.5+/-3.3), and more disabled (LPADL M=11.4+/-1.4 vs. M=7.0+/-3.1) (all p<.05). Linear regression examined the impact of education on 3MS and 2-month LPADL, adjusting for age, sex, history of dementia, and in-hospital delirium, as well as pre-fracture LPADL for the 2-month LPADL regression. Education was positively associated with 3MS scores (b=1.0(0.3), p<.01, Model R-squared=0.33), as well as 2-month post-fracture functional outcomes (b=-0.2(0.1), p< .05, Model R-squared=.35). Our findings indicate that education is associated with cognitive functioning and recovery in physical functioning following a hip fracture.

49. Theory Based Smart Phone Intervention for Aging Population with Type 2 Diabetes Mellitus (T2DM) to Enhance Health Behavioral Adherence

Kyungsook Kim

Poster Presentation; Room 349;
Informatics/Policy/Social Science 2

Prevention of complications through effective management is critical for well-being of the aging population with T2DM. A modified diet and regular physical activity are known to benefit blood glucose and lipid profiles, decrease the risk of end-organ damage, and improve psychological outcomes. Despite these benefits, lack of adherence to lifestyle change among T2DM aging population is a known problem. Research has shown that individuals with higher self-efficacy were more likely to adhere to medications, eat healthy, exercise more, and monitor blood glucose. The quality of patient-provider interaction has

also been shown to be associated with adherence to T2DM management. Little research has investigated interventions motivating improved self-efficacy and enhancing behavioral adherence.
A review of the theoretical literature suggests that interventions combining patient-centered with provider-interaction are the most powerful ones for T2DM. This suggests that targeted patient-centered interventions need to emphasize increasing self-efficacy (efficacy expectations and outcome expectations) and patient-provider interaction (communication regarding screening, treatment recommendations, diet, glucose monitoring, and physical activity) in order to enhance behavioral adherence.
A smart phone intervention based on self-determination and social cognitive theories is a potential avenue to autonomous motivation for self efficacy and behavioral adherence through patient-provider interaction. Electronic medical records of providers will generate automated tailored voice messages for patients regarding follow-up screening, treatment recommendations, and reminders for healthy behavior. The outcome can be measured with biological factors such as hemoglobin A1c, blood pressure, and LDL-cholesterol.

50. Access to Care, Race and Education Are Key Determinants of Erythropoietin Stimulating Agent (ESA) Use In Myelodysplastic Syndromes (MDS)

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Poster Presentation; Room 349; Informatics/Policy/Social Science 2

Background: ESAs are recommended by the National Comprehensive Cancer Network for low-risk MDS patients with symptomatic anemia who have low serum erythropoietin (Epo) levels and limited transfusion burden. Because ESAs are costly and involve repeated physician office visits, socioeconomically vulnerable patients may be less likely to receive them. The advent of reporting MDS to the Surveillance Epidemiology and End Results (SEER) registries as a cancer beginning in 2001 affords the ability to examine population-based utilization of therapies for this group of disorders. **Data and Methods:** Medicare patients diagnosed with MDS from 2001-2005 were identified in SEER registries. Medicare claims provided detailed treatment-related data on ESA use. Bivariate analyses and multivariate logistic regressions examined the effects of patient demographic, socioeconomic, geographic and health status characteristics, measured during 12 months prior to MDS diagnosis, on the probability of receiving ESAs between the year prior to SEER-reported diagnosis and either death or censoring. Analyses examined all MDS patients, and a subset with lower-risk MDS (modified French-American-British categories of refractory anemia (RA), RA with sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD), or del(5q) syndrome). **Results:** The MDS sample included 7,385 patients, with 2,568, or 35%, identified as lower-risk. 66% of MDS patients (70% of lower-risk) received ESAs at some point during the observation period. Multivariate estimates indicated that ESA use rates were higher among patients with an FAB classification of RARS relative to RA (Odds ratio [OR] 1.27; 95% Confidence interval [CI] 1.02-1.57), a history of transfusions prior to MDS diagnosis (OR 1.82; CI 1.58-2.09), diagnosis in 2004 versus 2001 (OR 1.21; CI 1.02-1.43), and residence in the South (OR 1.36; CI 1.12-1.65) or West (OR 1.28; CI 1.06-1.56) compared to the Northeast. Lower ESA use rates were also associated with baseline health status measures, including a diagnosis of dementia (OR 0.62; CI 0.51-0.76), other severe mental illness (OR 0.54; CI 0.36-0.83), use of a wheelchair

(OR 0.71; CI 0.58-0.88) or nursing home stay (OR 0.40; CI 0.30-0.53), and demographic and socioeconomic characteristics including age 85+ compared to age 65-69 years (OR 0.76; CI 0.61-0.95), black versus white (OR 0.77; CI 0.62-0.97), prior-year enrollment in Medicaid or Medicare Savings Programs (MSP) (OR 0.63; CI 0.53-0.75), and area percent of adults without any college education (OR 0.99; CI 0.98-1.00, p<.001). Among the lower-risk patients, the effects of RARS category, prior period transfusions, dementia, wheelchair use or nursing home stay, prior period Medicaid, and region of the country were similar to those in the full cohort. Lower-risk males were less likely than females (OR 0.76; CI 0.61-0.94) to receive ESAs. Diagnosis year and race were not significant factors in lower-risk patients (OR 0.96; p=0.84 for blacks compared to whites). **Conclusions:** Access to care, as reflected by Medicaid/MSP enrollment, race, and low educational attainment are key determinants of ESA use. Patient mobility and cognitive ability are also associated with reduced use. **Implications:** These data suggest that the current health care system may induce disparities in access to potentially important palliative care for patients with MDS. Ongoing research is examining the relationship between physician characteristics and receipt of ESA. Strategies to improve access should be considered, including changes to Medicare coverage of ESAs that could facilitate home administration. Acknowledgements: This project is funded through NIH/NCI RC1 CA145831-01.

51. Regulation of the Matriptase-Prostasin Cell Surface Proteolytic Cascade by Hepatocyte Growth Factor Activator Inhibitor-1 during Epidermal Differentiation

Ya-Wen Chen, Jehng-Kang Wang, Feng-Pai Chou, Chiu-Yuan Chen, Ellen A. Rorke, Li-Mei Chen, Karl X. Chai, Richard L. Eckert, Michael D. Johnson, and Chen-Yong Lin

Poster Presentation; Room 349; Basic Science 6

Matriptase, a membrane-tethered serine protease, plays essential roles in epidermal differentiation and barrier function, largely mediated via its activation of prostasin, a glycosylphosphatidylinositol-anchored serine protease. Matriptase activity is tightly regulated by its inhibitor hepatocyte growth factor activator inhibitor-1 (HAI-1) such that free active matriptase is only briefly available to act on its substrates. In the current study we provide evidence for how matriptase activates prostasin under this tight control by HAI-1.Whenprimaryhumankeratinocytes are induced to differentiate in a skin organotypic culture model, both matriptase and prostasin are constitutively activated and then inhibited by HAI-1. These processes also occur in HaCaT human keratinocytes when matriptase activation is induced by exposure of the cells to a pH 6.0 buffer. Using this acid-inducible activation system we demonstrate that prostatin activation is suppressed by matriptase knockdown and by blocking matriptase activation with sodium chloride, suggesting that prostatin activation is dependent on matriptase in this system. Kinetics studies further reveal that the timing of autoactivation of matriptase, prostasin activation, and inhibition of both enzymes by HAI-1 binding are closely correlated. These data suggest that, during epidermal differentiation, the matriptase-prostasin proteolytic cascade is tightly regulated by two mechanisms: 1) prostasin activation temporally coupled to matriptase autoactivation and 2) HAI-1 rapidly inhibiting not only active matriptase but also active prostasin, resulting in an extremely brief window of opportunity for both active matriptase and active prostasin to act on their substrates.

52. THE ROLE OF PERIORAL SENSATION DURING SUCKLING AND SWALLOWING IN INFANTS.

Shaina Holman, Stacey Lukasik, Regina Campbell-Malone, Peng Ding, Rebecca German

Poster Presentation; Room 349; Basic Science 5

Objective: Perioral sensation plays a key role during food acquisition, however, its role in normal suckling and swallowing is unknown. We wish to determine the precise role of perioral sensation during food transport in the oral cavity as well during the pharyngeal swallow.
Methods: Mammalian feeding was studied using an infant pig model. On two successive days, each individual either had (1) bilateral local infiltrations of bupivicaine to the lips or (2) no treatment control. Infant pigs were was bottle-fed milk containing barium while recording videofluoroscopy at 30 fps. We collected one feeding sequence every three hours following each treatment for a total of three feedings each day. We analyzed suck cycle duration, suck-swallow cycle duration rate and suck cycles per swallow with analysis of variance (ANOVA) to determine the effect of each treatment on feeding ability.
Results: The results showed that there was a significant decrease in suck cycles per swallow for the animals with anesthetized lips as compared to the control ($p<0.000$). The suck cycle and suck-swallow cycle durations were not significantly different between the two treatments ($p=0.582$ and $p=0.121$ respectively).
Conclusions: Lip infiltrations of local anesthesia caused animals to suck more often prior to a swallow although the duration of the suck and suck-swallow cycles did not change. This suggests that perioral sensation can affect the timing of swallow initiation. Further studies will examine the impact of other fields of trigeminal sensation on pharyngeal function.

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