UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS

22ND ANNUAL STUDENT RESEARCH FORUM

and

STUDENT RESEARCH AWARDS CONVOCATION

GRADUATE SCHOOL
SCHOOL OF MEDICINE
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JANUARY 16, 2008
ANSCHUTZ MEDICAL CAMPUS
RESEARCH COMPLEX 1
2ND FLOOR ATRIUM

22ND ANNUAL
UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS
STUDENT RESEARCH FORUM

Wednesday, January 16, 2008

Poster Session
1:30-3:30 pm

Awards Convocation
4:00 pm

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STUDENT RESEARCH FORUM
JANUARY 16, 2008

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ABBOTT, SM

CONVERSION-DEPENDENT STRESS RELAXATION IN CROSSLINKED POLYMERS. SM Abbott (DDS, University of Colorado School of Dental Medicine), J Garcia, SM Newman, J Stansbury.

One of the most clinically significant problems associated with current polymer-based composite materials is the stress that develops at the restorative-tooth interface during their polymerization. This challenge dictates that detailed bonding protocols and restorative placement procedures be followed to allow successful clinical outcomes. A tremendous amount of research activity has been directed towards new restorative materials designed to produce lower stress dental composites or to the measurement of stress in commercial and experimental materials. However, little attention has been focused on improving the fundamental understanding of stress development, which includes stress relaxation as an integral component. Objective: Therefore, the goal of this investigation was to examine how the stress relaxation rate varies with the extent of polymer formation.

Methods: Polymer specimens (n=16) composed of Bis-GMA and triethylene glycol dimethacrylate (TEGDMA) (7:3 weight ratio) were obtained by UV irradiation with exposure intervals varied from 5 to 330 s. Tetraethylthiuram disulfide was used as a photo-iniferter to produce chemically and thermally stable partially cured polymers as 1x2x25 mm beam specimens. The degree of conversion in each specimen was measured by near-infrared spectroscopy followed by evaluation of modulus and stress under constant strain conditions in three-point bending within the linear elastic limit on a universal testing machine.

Results: Photopolymer specimens with stable conversion values ranging from <10% to >50% were obtained. Both the modulus calculated from the initial deflection loading and the peak stress corresponding with ending position held throughout the stress relaxation evaluation increased in an exponential manner with increasing conversion. The normalized residual stress values at 1, 10 and 100 s were 99, 93 and 80 %, respectively, for the high conversion, high modulus polymers compared with residual stress values of 93, 78 and 53 % for the low conversion material held over the same time intervals. A linear dependence was observed between polymer modulus and the normalized residual stress level with r^2 correlations of 0.77, 0.87 and 0.90, respectively, for the data taken at 1, 10 and 100 s.

Conclusions: While only minimal stress levels can be supported in low conversion polymers due to their low moduli, substantial percentages of stress are retained over extended time periods even for polymers only slightly beyond gel point. Despite the much higher absolute stress levels possible in polymers at high conversion, the potential for stress recovery decreases considerably with increasing polymerization. This information will assist in understanding and modeling the complex dynamic stress evolution process in bonded dental composite restorations.

ANGLES, EM

RISK FACTORS FOR DELIRIUM FOLLOWING MAJOR TRAUMA. EM Angles, (M.D., SOM), TN Robinson, J Johnson, WL Biffl, M Moss, ZV Tran, and EE Moore, Department of Surgery, University of Colorado.

Purpose: Delirium is an acute, fluctuating cognitive dysfunction triggered by physiologic stress. Delirium is common following major trauma, and has been associated with worse outcomes, especially among elderly patients. The objectives of this study were to

identify risk factors that predict the development of delirium in trauma patients and to compare outcome measures in subjects with and without delirium.
Methods: A prospective observational study was performed on patients with an Injury Severity Score (ISS)>8 admitted to the trauma intensive care unit (ICU) of a major level I trauma center. After written informed consent was obtained, subjects were evaluated daily for delirium using validated tools including the Richmond Agitation Sedation Scale (RASS) and the Confusion Assessment Method-ICU (CAM-ICU). The CAM-ICU defines delirium as present or absent by evaluating the level of consciousness (RASS score) in combination with inattention and disorganized thinking. Univariate and multivariate analyses were performed and reported as mean±SEM.
Results: During 394 total ICU days, 69 patients (55 male) were studied. Delirium occurred in 41 (59%) subjects. Delirium presented most frequently on post-injury day 2 with a duration of 4.7±0.7 days. Delirium-related events occurred in 7 (17%) subjects, including self-extubation and patient removal of lines and tubes. Univariate analysis determined the following variables to be associated with developing delirium: older age (48±3 vs. 37±3 years, $p=0.03$), higher ISS (26±2 vs. 20±2 days, $p=0.02$), lower arrival Glasgow Coma Score (GCS) (12±1.0 vs. 15±0.1, $p<0.01$), increased units of blood transfusion (2.8±0.7 vs. 0.5±0.3, $p<0.01$), higher multiple organ failure (MOF) score (1.2±0.2 vs. 0.1±0.1, $p<0.01$) and more operations (1.3±0.2 vs. 0.4±0.1, $p<0.01$). Multivariate analysis determined arrival GCS, increased blood transfusion requirement and higher MOF score to be the strongest predictors for developing delirium. Outcome measures revealed subjects with delirium had more complications (49% vs. 7%, $p<0.01$), longer ICU stays (7.8±1.1 vs. 2.1±0.2 days, $p<0.01$) and longer hospital stays (15.2±1.7 vs. 6.1±0.7 days, $p<0.01$).
Conclusions: Delirium occurs in over half of patients admitted to the ICU with major trauma. Age and arrival GCS were the two pre-ICU admission variables which predicted the development of delirium. The overall strongest predictors of developing delirium following major injury were higher arrival GCS, increased transfusion requirement and increased MOF score. Subjects with delirium have worse outcomes including increased complications, longer ICU stays and longer hospital stays.

BADTKE, MM

PROGESTERONE RECEPTORS PROTECT BREAST CANCERS FROM KILLING BY TAXANES. MM Badtke, (Ph.D., GS), P Jambal, BM Jacobsen and KB Horwitz
Department of Medicine, University of Colorado Denver – Anschutz Medical Campus, Aurora, CO, USA 80045

Introduction: The taxanes (Tx), paclitaxel (Px) and docetaxel (Dx), are among the most effective treatments for advanced breast cancers. While both taxanes kill cancer cells, clinical studies suggest that Dx is more effective than Px in inducing apoptosis and is often effective in Px resistant cancers. Response to taxanes is influenced by presence of estrogen (ER) and/or progesterone (PR) receptors in tumors. Using ER+ breast cancer cells, we find that expression of PR generates resistance to Px-induced apoptosis.

Study Objectives: To 1) define molecular mechanisms by which ER and PR suppress tumor response to taxanes, 2) to define the mechanisms underlying differences in response to Dx. *vs.* Px; and 3) to identify the genes regulated by taxanes in breast cancers.

Methods: To determine possible mechanisms of PR mediated resistance to taxane induced apoptosis, expression profiling was performed in ER+ breast cancer cells with inducible PR.

Cells either lacking or expressing PR for 48h were treated with vehicle, Px or Dx for 24h. This allowed us to define genes regulated by taxanes in the absence or presence of PR.

Results: By gene expression profiling, the total number of genes regulated by Px and Dx is similar, and many genes are the same, but, a subset of genes are uniquely regulated by each Tx. Four classes of genes are regulated by both Tx and further regulated by PR: Class I) are upregulated by Tx and downregulated by PR; Class II) are upregulated by Tx that are further upregulated by PR; Class III) are downregulated by Tx and upregulated by PR; and Class IV) are downregulated by Tx and further downregulated by PR. Many of the genes oppositely regulated by Tx and PR control proliferation, cellular metabolism, and apoptosis.

Conclusion: Key genes for both Tx modulate apoptosis. PRs regulate multiple genes, some of which overlap with Tx regulated genes. As a result, the presence of PRs modifies the ability of both Px and Dx to regulate gene expression. We continue to study the mechanisms by which PRs generate resistance to Tx-induced apoptosis.

BAUERLE, KT

EFFECT OF INHIBITION OF MEK1/2-DEPENDENT NFKB ACTIVITY ON THYROID CANCER CELL PROLIFERATION. KT Bauerle (MD, PhD), RE Schweppe, and BR Haugen, Division of Endocrinology, Department of Medicine, University of Colorado, Denver, CO.

Papillary thyroid cancer (PTC) accounts for ~80% of all thyroid cancer cases. Approximately 30% of all cancers have aberrant activity in the MAP kinase pathway. Conversely, 70% of PTC cases have constitutive MAPK activity (primarily $BRAF^{V600E}$ point mutation and RET/PTC1 translocation). NFkB can promote survival, angiogenesis, invasion, and proliferation in many cancers. In this study, we examined the effect of MAPK signaling on NFkB activity and the role of NFkB in thyroid cancer. We first transfected an NFkB-responsive luciferase reporter plasmid into two rat thyrocyte cell lines (PCCL3) each containing a stably integrated $BRAF^{V600E}$ or RET/PTC1 cDNA under the control of an inducible promoter. Induction of either oncogene increases NFkB activity, indicating that MAPK signaling by either oncogene can induce NFkB signaling. Furthermore, pharmacologic inhibition of MEK1/2 demonstrates that the NFkB activity is dependent on MEK1/2 activity. While 50% inhibition of NFkB activity is observed in cells expressing $BRAF^{V600E}$, only 25% inhibition of NFkB activity is observed in cells expressing RET/PTC1, suggesting differential dependence on MEK1/2 signaling to NFkB by these two oncogenes. To evaluate the role of these proto-oncogenes on NFkB activity in thyroid cancer cells, we examined two PTC lines, 2-7 BHP and 5-16 BHP, which harbor the RET/PTC1 translocation and the $BRAF^{V600E}$ mutation, respectively. While NFkB activity present in these cell lines was inhibited by ~50% in response to overexpression of a kinase-dead MEK, pharmacologic inhibition of MEK1/2 produced a decrease in activity in only 5-16 BHP cells. Finally, we used an NFkB inhibitor (Bay 11-7082) to study proliferation of a panel of ATC and PTC cell lines by Vi-Cell counting. While ≥50% inhibition was observed in four cell lines (TPC1, 5-16 BHP, NPA, and BCPAP), two cell lines (ARO and K1) appear to be more resistant (<70% inhibition). Further studies are necessary to elucidate the differential response of these cell lines to the NFkB inhibitor because it is apparent that response to the drug is independent of mutational status. In summary, we show that 1) NFkB activity is induced by oncogene overexpression, 2) NFkB activity is dependent on MEK1/2 activity, and 3) NFkB inhibition decreases cell proliferation. In conclusion, these findings suggest that NFkB is an appropriate target for future thyroid carcinoma therapeutics.

BENNETTS, NA

IMPORTANCE OF THE BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTOR, HAND2, IN CRANIOFACIAL DEVELOPMENT. NA Bennetts (DDS, SOD), Francie Hyndman[1], Marthe Howard[2] and DE Clouthier[1], [1]Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Denver, Aurora, CO. [2]Department of Neurosciences, Medical College of Ohio, Toledo, OH.

Proper development of craniofacial tissues depends largely upon the formation, migration, differentiation, and survival of cranial neural crest (CNC) cells during embryogenesis. These pluripotent cells migrate away from the hindbrain neuroepithelium around the time of neural tube closure, coming to rest in the pharyngeal arches, transient structures on the embryo ventral surface. Within the first mandibular pharyngeal arch, their communication with surrounding tissues (i.e. epithelial ectoderm, paraxial mesoderm, and pouch endoderm) set up hierarchical signaling cascades, mediated by transcription factors, that direct correct spatiotemporal formation of the adult facial skeleton. **Objective:** To understand the mechanism by which the basic helix-loop-helix (bHLH) transcription factor Hand2, expressed in the distal mesenchyme of the mandibular arch, regulates facial development. While loss of Hand2 in zebrafish leads to craniofacial defects, loss of Hand2 in mice leads to early embryonic lethality from vascular defects, precluding further analysis. **Methods:** To address the function of Hand2 in mammalian development, we have used Cre-*lox*P technology to create *Hand2$^{fl/fl}$;Wnt1-Cre* embryos in which the *Hand2* gene is inactivated in CNC cells, permitting further analysis of its function. We sought to characterize craniofacial deformities in *Hand2$^{fl/fl}$;Wnt1-Cre* embryos using hematoxylin and eosin-stained sagittal sections of E18.5 embryos. Furthermore, we examined the expression of two potential Hand2 effectors, *Goosecoid* and *Msx1,* via whole mount in situ hybridization using E10.5 embryos. **Results:** We find that *Hand2* mutants exhibit many lower jaw defects including mandibular hypoplasia, absence of the submandibular gland and tongue and the presence of maxillary soft tissue features in the mandible. Furthermore, *Goosecoid* expression in *Hand2* mutants is downregulated while *Msx1* is unaffected. **Conclusions:** These findings suggest that Hand2 promotes distal lower jaw formation through maintenance of distal gene expression, with loss of function resulting in a spread of maxillary fates into the distal domain. Support: NIH/NIDCR DE14181.

BOWERS, E

IMPAIRED IMMUNOGLOBULIN GENE (V_H3) SOMATIC HYPERMUTATION AND ACTIVATION-INDUCED CYTIDINE DEAMINASE EXPRESSION DURING HIV INFECTION. E Bowers (Ph.D., GS), KM Keays, L Beniguel, A Asrani, S MaWhinney, BE Palmer, AR Bajar, JR Thurn, RW Scamurra, and EN Janoff. Department of Infectious Diseases, Colorado CFAR, University of Colorado, Denver, CO; and MAVRC, University of Minnesota, VAMC, Minneapolis, MN.

Background: Control of invasive bacterial infections by antibodies is related to somatic hypermutation (SHM) of variable regions of immunoglobulin heavy chain genes (VH). SHM is mediated by the B-cell-specific molecule, activation-induced cytidine deaminase (AID). Methods: We analyzed 1,565 cDNA IgG B-cell V_H3 sequences from 10 uninfected control subjects (Cont), and 21 HIV-infected patients (CD4 count <400) (15 untreated [HIV], 6 treated with undetectable plasma HIV RNA [HIV-Rx]). We performed immunophenotyping by flow cytometry and AID expression by quantitative RT-PCR of

PBMC stimulated with anti-IgM, anti-CD40, and IL-4 from 22 Cont, 19 HIV-Rx and 16 HIV subjects. We used non-parametric tests, and for 3 group comparisons with p<.05, pairwise tests with Bonferroni correction. Results: Mutation frequencies in antigen-binding hypervariable CDR1/2 regions were lower among HIV vs. Cont for nucleotides (median 10% vs. 13.5%; p=.043) and amino acids (20% vs. 25%; p<.05), but not structural framework regions. Expression of AID (□CT AID/□-actin) was >1 log higher in HIV (p=.002) and HIV-Rx (p<.05) on day 0 vs. Cont. Although stimulation increased AID expression in all groups, the fold-increase was lower in HIV (p<.0001) and HIV-Rx (p=.004) vs. Cont. In unstimulated CD19+ B-cells, activation levels were higher ($CD86^{+}CD21^{-}CD23^{-}$, p<.02) in HIV vs. Cont. CD4+ and CD8+ T-cell activation ($CD38^{+}HLA\text{-}DR^{+}$) was also higher among HIV (p<.0008). HIV-Rx showed intermediate values. Conclusions: Increased baseline levels of both B- and T-cell activation, were associated with decreased mutation frequencies in antigen-binding V_H3 CDR genes and levels of the critical SHM-inducing AID in B-cells from HIV patients. However, the ability of B-cells from HIV to respond to stimulation was significantly decreased vs. Control subjects. The lower levels of SHM and the decreased ability to generate AID in response to stimulation may underlie the increased risk and impaired humoral responses to opportunistic infections and vaccines designed to prevent them.

CANNON, CMA

CHARACTERIZATION OF AML CELL LINES FOR EXPRESSION OF AXL AND MER.
CMA Cannon, MD candidate in the School of Medicine, AK Keating and DK Graham, Department of Pediatrics, University of Colorado, Denver, CO.

INTRODUCTION: Leukemia is the most common pediatric malignancy, afflicting 6,500 kids a year in the United States alone. Many types of leukemia are treatable, but less than half of children afflicted with the Acute Myelogenous Leukemia (AML) type will survive. The development of therapies targeted more specifically at myeloid leukemia cells offers the potential for improving AML survival rates while reducing toxicity and secondary cancer risks. I hypothesized that AML cell lines express Mer and Axl tyrosine kinases and that this expression may play a causative role in leukemogenesis.

PURPOSE: To identify AML cell lines expressing Axl and Mer. The identification of Mer and Axl expression will allow us to further investigate these tyrosine kinases and their role as future therapeutic targets for AML.

METHODS: Ten AML cell lines were grown *in vitro* and characterized for Axl and Mer expression using flow cytometry and Western blot techniques. The following cell lines were investigated: Molm-13, KG-1, KG-1A, HEL, EOL-1, THP-1, Nomo-1, and HL-60. U937 cells, known to express Mer, were used as a positive control for Mer. A549 cells, known to express Axl, were used as a positive control for Axl.

RESULTS: Axl expression was found to be positive in the KG-1A cell line. KG-1 cells, a further differentiated form of the KG-1A cell line, and Nomo-1 cells were positive for expression of Mer. The HEL cell line expressed both Axl and Mer. Molm 13, THP-1, EOL-1, and HL-60 cells lines were all negative for both Axl and Mer expression.

CONCLUSIONS: Axl and Mer are expressed by a subset of AML cell lines. Further investigation of these cell lines using downregulation and inhibition of Axl and Mer expression will help to identify possible therapeutic targets allowing individualized treatment of AML resulting in lower toxicities and secondary cancer risks.

CAPORASO, JG

EVALUATING COEVOLUTION DETECTION METHODS ON PROTEIN ALPHA HELICES.

J. Gregory Caporaso, Lawrence Hunter, and Rob Knight

Correlated evolution (coevolution) between positions in biological sequences is a source of important information about biomolecules including structural, allosteric, and intermolecular interactions. Coevolutionary data is however, difficult to acquire due to the stochastic nature of sequence evolution, varying degrees of relatedness between sequences in input alignments, the multiple-comparisons problem involved in analyzing all pairs of positions in alignments, and in proteins in particular, the lack of biologically relevant evaluation data. We propose using alignments of alpha helical proteins to address the lack of evaluation data for judging coevolution detection methods. Ionic interactions between stacked residues in alpha helices are known to be important for alpha helix stability, and we therefore expect these positions to coevolve. We applied a variety of automated methods for detecting coevolution to alignments of alpha helices, including tree-ignorant methods (which are generally fast, but not theoretically robust) and tree-aware methods (which are more theoretically robust, but generally slow). We additionally experimented with 52 reduced amino acid alphabets, defined based on different characteristics of amino acids and with varied alphabet sizes. We observe coevolutionary signal between positions 3, 4, and 7 residues apart, coinciding with the periodicity of the alpha helix. Alpha helices therefore provide a biologically relevant data set for evaluating and comparing high-throughput methods for detecting protein coevolution.

CHECKETTS, M

INDUCTION OF CELL DEATH BY RECEPTOR EXPRESSED IN LYMPHOID TISSUE, A RECENTLY IDENTIFIED TUMOR NECROSIS FACTOR RECEPTOR. M. Checketts[1] (DDS, SOD), M. Reyland[1], H. B. Shu[2], and J. Cusick[1]. University of Colorado School of Dentistry[1]. College of Life Sciences, Peking University, Beijing, China[2].

Tumor Necrosis Factor Receptor (TNFR) family members induce a variety of cellular responses including apoptotic cell death and are implicated in a variety of human diseases including cancer, inflammation and periodontal disease. RELT (Receptor Expressed in Lymphoid Tissues) is a recently identified member of this family whose function is largely unknown. Previous results suggest that RELT induces cell death in human epithelial cells. **Objectives:** To define the portion of the RELT protein required to induce cell death in human epithelial cells. **Methods:** The ability of different RELT deletion mutant constructs to induce cell death in human epithelial cells was examined. Mutagenesis of the RELT gene using site-directed mutagenesis followed by transformation of mutated plasmid constructs into *E. coli* cells was performed. Plasmid DNA was isolated from *E. coli* clones and automated DNA sequencing was utilized to verify creation of mutant constructs. Transient transfection of HEK-293 cells was used to examine the effect of expressing mutant RELT constructs in a human epithelial cell line. Cell death was assayed by both X-gal staining and TUNEL staining to detect fragmented DNA. Expression of wild-type RELT and Caspase-8 were used as positive controls, expression of empty plasmid vector was used as a negative control. Western blots were used to confirm expression of mutant protein constructs. **Results**: Four intracellular deletion mutants of RELT were successfully created that were used in combination with previous collection of RELT deletion mutants. The expression of most proteins was verified. The mutant protein constructs retained varying abilities to induce apoptosis morphology and DNA fragmentation in HEK cell line.

Conclusion: Expression of RELT in HEK-293 cells induces apoptotic like morphology and DNA fragmentation supporting previous results. Preliminary results suggest that there may not be one single intracellular domain of RELT responsible for inducing cell death. Support: NIH/NIDCR U24 DE016502

CHONG, B

INTERACTION BETWEEN SPLICEOSOME PROTEINS PRP8, BRR2 AND SNU114. Brandi Chong, (Ph.D., GS), L. Zhang and R. Zhao, Department of Biochemistry and Molecular Genetics, University of Colorado, Denver, CO.

Pre-mRNA splicing is essential for gene expression in all eukaryotes. Pre-mRNA splicing is catalyzed by the spliceosome, a huge RNA-protein complex containing over 100 different proteins and 5 small nuclear RNAs (snRNAs). Prp8, Brr2, and Snu114 are proteins of the U5 snRNP that are thought to regulate pre-mRNA splicing. Prp8 is one of the largest and the most conserved proteins in the nucleus. It is the only spliceosomal protein that extensively cross-links with 5' and 3' splice sites and the branch point. Prp8 is thought to play a critical role in forming and stabilizing the catalytic core. Brr2 is a helicase with ATPase activity that unwinds the U4/U6 snRNA, an essential step for the activation of the spliceosome. Snu114 is the only GTPase required for splicing and its specific function in splicing remains unclear. Our lab recently determined the crystal structure of the Prp8 C-terminal domain (CTD) and proposed that the CTD interacts with Brr2 and Snu114. In my project, I studied the interaction among the CTD of Prp8, Brr2, and Snu114. I also obtained diffracting crystals of a unique domain of Brr2. These studies lay the foundation for understanding the structure and function of Prp8, Brr2, and Snu114.

CHOUDHRY, IK

BIAS TOWARDS PUBLISHING POSITIVE RESULTS IN ORTHOPEDIC AND GENERAL SURGERY: A PATIENT SAFETY ISSUE? Hasenboehler, EA; Choudhry, IK (M.D., SOM), Newman, JT; Smith, WR; Ziran, BH; Stahel, PF. *Patient Safety in Surgery* 2007, **1**:4.

BACKGROUND: Research articles reporting positive findings in the fields of orthopedic and general surgery appear to be represented at a considerably higher prevalence in the peer-reviewed literature, compared to published studies on negative or neutral data. This "publication bias" may alter the balance of the available evidence-based literature and may affect patient safety in surgery by depriving important information from unpublished negative studies. METHODS: A comprehensive review of all published articles in a defined 7-year period was performed in 12 representative journals in the fields of orthopedic and general surgery. Every article published in all volumes of these journals between January 2000 and December 2006 was reviewed and rated by three investigators. Rating of articles was performed according to a uniform, standardized algorithm. All original articles were stratified into "positive", "negative" or "neutral", depending on the reported results. All non-original papers were excluded from analysis. RESULTS: A total of 30,197 publications were reviewed over a 7-year time-period. After excluding all non-original articles, a total of 16,397 original papers were included in the final analysis. Of these, 12,251 (74%) articles were found to report positive findings, 2,709 (17%) reported negative results, and 1,437 (9%) were neutral. A similar publication pattern was found among all years and all journals analyzed. Altogether, 91% of all original papers reported significant data (positive or negative), whereas only 9% were neutral studies that did not report any significant findings. CONCLUSION: There is a disproportionately high number

of articles reporting positive results published in the surgical literature. A bias towards publishing positive data will systematically overestimate the clinical relevance of treatment effects by disregarding important information derived from unpublished negative studies. This "publication bias" remains an area of concern and may affect the quality of care of patients undergoing surgical procedures.

CHOUDHRY, IK

CYTOKINES IN LOW BACK PAIN. IK Choudhry (M.D., SOM),D Solomonow, E Roper, KB King, and M Solomonow. Bioengineering Division, Department of Orthopaedics, University of Colorado, Denver, CO.

PURPOSE: Prolonged exposure to repetitive loading in an occupational setting (such as movers, warehouse workers, dock loaders, etc.) leads to chronic low back dysfunction characterized by pain, weakness, stiffness, and decreased range of motion, a condition termed cumulative trauma disorder (CTD). Ligamentous creep (laxity) through repetitive microtrauma has been shown to contribute to CTD. Although the electromyographic data provide evidence for inflammation within the viscoelastic tissues of the lumbar spine after repetitive loading, the molecular characteristics of this process have not been elucidated. The purpose of this study was to measure proinflammatory cytokines in an animal model for low back pain. METHODS: Nine cats were anesthetized after which the back was dissected and an s-hook inserted beneath the L4-5 supraspinous ligament. The L4-5 ligament was cyclically loaded for 10 minutes with a 40 N load at a frequency of 0.25 Hz followed by 10 minutes of rest repeated six times. The loading/rest period was followed by a 7-hour recovery at rest. Experimental L4-5 ligaments and control T10-11 ligaments were excised and flash frozen. RNA was then extracted from powdered ligaments. The RNA was treated with DNase and reverse transcribed. Finally, the cDNA was analyzed using quantitative real-time PCR. RESULTS: Cytokine levels in the experimental L4-5 ligament normalized to the housekeeping gene GAPDH were compared to the control T10-11 ligament using a paired two-way Students' t-test. Three of the five cytokines analyzed were more highly expressed in the loaded L4-5 ligament. These included IL-1B ($P=0.07$), IL-6 ($P=0.01$), and IL-8 ($P=0.02$). The other two, TGF-B and TNF-a, had minimal expression and showed no significant change in expression. DISCUSSION: The results clearly indicate that cytokines play an active role in the damage/repair mechanisms that occur in ligaments following repetitive trauma. The particular correlation between IL-1B, IL-6, and IL-8 has been noted before in studies of various arthropathies. However, the exact relationship between these cytokines remains speculative. Based on what is known about the mentioned cytokines, it appears that IL-1B expression induces expression of IL-6 and IL-8 through T-cell activation. This relationship is certainly consistent with our results. It is well known that IL-1B antagonism can clinically reduce the inflammatory response in certain autoimmune arthropathies. Such research has allowed successful treatment of diseases using cytokine specific antagonists. IL-6 blockade has also gained interest as a potential therapeutic target. Together IL-1B, IL-6, and IL-8 contribute as inflammatory mediators through a complex interplay. Finding the exact role these cytokines play in ligamentous low back disorders is vital to developing therapeutic targets for this common ailment.

CIRONA, LA

EVALUATING THE EFFICACY OF APPETITE AWARENESS TRAINING IN THE TREATMENT OF EATING DISORDERS IN A MILIEU-BASED PROGRAM: A PILOT STUDY. LA Cirona. (MD program), Jennifer Hagman (sponsoring faculty), Cinda Nab, and Mindy Solomon, Department of Psychiatry, The Children's Hospital, Denver, CO.

Appetite dysregulation is a common feature of eating disorders (EDs). Appetite awareness training (AAT) is a form of therapy designed to address this feature of ED psychopathology by increasing interoceptive awareness of hunger and fullness. It has been shown to be an effective treatment in adult women with binge eating disorder and bulimia nervosa, but has not yet been evaluated in an adolescent population, milieu based programs, or individuals with anorexia nervosa. AAT involves attending a weekly group and completion of daily appetite monitoring forms in which individuals rate their satiety level prior to and after eating. The purpose of the present study was to evaluate the efficacy of AAT in the ED Treatment Program, a milieu-based setting, at The Children's Hospital in Denver. There were five outcome measures, three of which were collected at baseline and at four weeks (Mizes Anorectic Cognition Scale; Interoceptive Awareness Questionnaire; and the Preoccupation with Eating, Weight, & Shape Scale); the other two were completed daily (appetite monitoring forms completed by the patients, and the Ease of Eating form, completed by staff). We hypothesized that participation in AAT would reduce ED thoughts and behaviors and increase awareness of and adherence to hunger and satiety cues. Demographic data are presented and feasibility of implementing AAT in a milieu based program is discussed.

CRAIG, D

CD5 CHARACTERISTICS DISTINGUISH T-CELL POPULATION SUBSETS IN DIABETIC MOUSE MODELS. Daniel Craig (MD, 2nd Yr), DH Wagner Jr., DM Waid, GM Vaitaitis, ND Pennock, Department of Medicine University of Colorado.

Type 1 Diabetes (T1D) is a disease of progressive destruction of pancreatic islet cells mediated by auto-aggressive T-cells. The identification and characterization of these self-reactive T-cells based on antigen specificity has proven difficult. However, by assaying for pathogenicity based on surface markers, recent research by Wagner et al has shown a subset of T-cells that are CD4lo and CD40+ and cause diabetes in mouse models. These same T-cells have been found in humans with T1D. Further research has also shown dis-regulation of T-cell populations in mouse models potentially leading to the expansion of a self-reactive T-cell population (CD4lo/CD40+). Thus the further characterization of those T-cells responsible for the dis-regulation and the inflammatory process proper may expose new methods for treatment or early diagnosis of the disease.

In this paper we describe a set of experiments designed as a broad survey of CD5 distribution and activity in the T-cell populations of diabetic and non-diabetic mouse models. CD5 is a surface protein found on most T-cells which mediates T-cell-receptor (TCR) signaling in co-ordination with Zap70 and CD3. It has also been shown to interact with downstream TCR cascade modulators Lck and Fyn. The following experiments show CD5 that CD5 distribution and activity vary significantly between strains of inbred mice. We also demonstrate here that regulatory T-cells (Tregs) ubiquitously express CD5 in every strain, and that Treg CD5 levels vary between strains. This paper is not an exhaustive explanation of CD5's role in diabetic models, but it does show that CD5 has the potential to be a significant modulator of the disease due to its unique distribution in diabetic mouse models compared to non-diabetic mice. This project is an invitation to further clarification of CD5's mechanism of T-cell modulation in CD4 helper cells as well as Tregs.

HOX GENE EXPRESSION MODULATES ANDROGEN- AND VITAMIN D-MEDIATED ACTIONS IN HUMAN PROSTATE CANCER CELLS

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HOX genes encode a large family of transcription factors involved in key developmental decisions, and are often aberrantly expressed in cancer. Our laboratory has previously shown that a subset of genes of the HOXC cluster are overexpressed in primary prostate tumors, metastases, and prostate cancer (PCa) cell lines[1]. Increasing transient expression of HOXC8 in LNCaP PCa cells as well as HPr-1 AR non-tumorigenic prostate epithelial cells results in a progressive suppression of androgen responsive promoters. Transcription from both the mouse probasin promoter and the MMTV promoter is inhibited at levels of HOXC8 expression comparable to those seen in PCa cell lines. Other members of the HOX family also inhibit androgen signaling. We have created LNCaP and HPr-1 AR derived lines that stably overexpress HOXC8 and show that signaling through androgen responsive promoters is inhibited, and PSA mRNA levels are decreased in these cell lines. HOX proteins block the histone acetyltransferase activity of the coactivators CBP and p300[2]. As these are key mediators of steroid-dependent transcription, inhibition of these coactivators could account for the HOX-dependent suppression of androgen receptor-mediated transcription. We show that overexpression of CBP relieves the inhibition of androgen receptor-mediated transcription by HOXC8. Further, chromatin immunoprecipitation demonstrates that HOXC8 expression inhibits hormone-induced histone acetylation at the androgen responsive MMTV promoter. HOXC8 overexpression has been shown to correlate with higher Gleason grade PCa[3]. Our preliminary studies demonstrate that stable overexpression of HOXC8 in HPr-1 AR cells increases both motility and invasiveness *in vitro*.

In contrast to androgens, the secosteroid vitamin D has been shown to have antiproliferative, prodifferentiation and antimetastatic properties in PCa. Increasing expression of HOXC8 also results in a progressive suppression of vitamin D-induced transcription in vitamin D-responsive ALVA-31 PCa cells. Interestingly, in LNCaP cells, overexpression of HOXC8 does the reverse. Here HOXC8 potentiates hormone-induced transcription from various vitamin D-responsive promoters.

These data indicate that the simple model of HOX overexpression impacting intracellular receptor activity through the inhibition of CBP/p300 is, at best, only part of the story. We propose that increased expression of HOXC genes during tumorigenesis precipitates a need for the tumor cells to overcome HOXC-mediated inhibition of androgen signaling, predisposing tumors cells to survive in the face of a subsequent androgen withdrawal. Understanding the mechanism underlying the failure of endocrine intervention could lead to the design of therapeutic modalities that would prolong the efficacy of androgen ablation therapy.

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FIER, J

USE OF BODY IMAGE SOFTWARE TO EVALUATE BODY IMAGE DISTORTION AND DRIVE FOR THINNESS IN PATIENTS WITH ANOREXIA NERVOSA. J Fier, (MD, SOM), J Hagman (MD), and M Dodge (BA), Children's Hospital, Denver, CO and The University of Colorado Health Science Center, Denver, CO.

Purpose: Body image distortion is a DSM-IV core diagnostic criteria for Anorexia Nervosa (AN). However, methods for measuring body image have historically been highly subjective. In the 1990's Body Image Software (BIS) was developed by Dr. Rick Gardner of the University of Colorado at Denver to improve the accuracy and precision of body image assessment. The software and subject directions have been adapted for the measurement of perception of current body size and drive for thinness, parameters thought to be correlated with AN severity. The purpose of this study was to analyze results from 3 different BIS tasks and to evaluate the use of this tool in subjects with AN. **Methods:** We conducted a review of current literature to compare advantages and limitations of BIS and other methods of body image measurement. We then analyzed BIS data from 3 different tasks for the first 47 subjects (females, age range 12-21) with AN enrolled in "A Double Blind, Placebo-Controlled Study of Risperidone for the Treatment of Anorexia Nervosa" (PI – Jennifer Hagman MD). The 3 BIS tasks take 15-30 minutes to complete. The tasks utilize a digital image of the subject which is then loaded into the software program. The subject is given standardized directions for each task, during which they work with their own distorted image on the screen. **Results:** The literature review illustrated that BIS software has not been widely used. However, it is the only modality we found that identifies and incorporates sensory differences into measurements of body image distortion. Consequently, BIS is likely a more accurate measure of body image distortion and drive for thinness. The mean age of subjects was 16 (min 12, max 21). The mean %Ideal Body Weight at the time of testing was 79.03% (min 64.60%, max 89.00%). The mean BIS Adjustment task for current body size was 9% larger than their actual body size and the mean BIS Adjustment task for desired body size was 8% less than their actual size. On the Adjustment Probit Estimation task (APE) the result for perceived current size was 7% larger than actual body size on average. Additionally, on average, patients can perceive a 2% change in body size. **Conclusion:** The Body Image Software is a useful tool in the study of AN. These preliminary findings suggest that subjects with AN do have body image distortion and drive for thinness. The finding that AN subjects are able to detect small changes in body size supports current thinking that sensory impairment is not responsible for body image distortion in AN patients. Furthermore, the BIS may be useful in determining if body size estimation improves in response to risperidone or other treatment interventions.

FREKING, AR

VOLTAGE-GATED SODIUM CHANNEL ISOFORM $Na_V1.7$ MAY BE PRESENT IN VASCULAR SMOOTH MUSCLE. AR Freking[1] (MS, DDS SODM), MM Tamkun[2], MA Henry[3], SR Levinson[4] University of Colorado Denver School of Dental Medicine, Aurora, CO[1]; Department of Biochemistry and Molecular Biology, Colorado State University, Fort

Collins, CO[2]; Department of Endodontics, University of Texas, San Antonio, TX[3], Department of Physiology and Biophysics, University of Colorado Denver, Aurora, CO[4]

Nine different genes encode for nine isoforms of voltage gated sodium channels (VGSCs) that are expressed in excitable tissues, and their distribution within the nervous system varies depending on the identity of the isoform. VGSCs play an essential role in the nervous system; they generate action potentials by allowing the entry of sodium ions into the cell during depolarization. Dental anesthetics like lidocaine are routinely injected into tissue to acutely block the activity of VGSCs in nerves supplying the gingiva and teeth, while great care is taken to not administer these drugs directly into systemic circulation. The high rate of failure of this common dental procedure has resulted in much dental research devoted to understanding the underlying mechanisms, physiology, and distribution of VGSCs. In previously published studies, we quantified a dramatic change in expression of VGSCs in a region located proximal to a lesion of the rat infraorbital nerve. In a second study using the same infraorbital nerve lesion model, we published evidence suggesting the specific involvement of the nodal isoform $Na_v1.6$ in this change of expression. While investigating the role of isoform $Na_v1.7$, we have made preliminary observations that $Na_v1.7$ is also found in vascular smooth muscular tissue. Identifying this isoform in cells previously thought to be free of $Na_v1.7$ would prove useful in accurately assessing the effect of anesthetic drugs like lidocaine on systemic circulation. **Purpose:** To show that $Na_v1.7$, a protein involved in nociceptive mechanisms, is also found in smooth muscle cells. **Methods**: Immunohistochemistry (IHC) and western blot analysis were used to test for the presence of $Na_v1.7$ in a variety of vascular tissue, including aorta, superior mesenteric, and femoral arteries. **Results and Conclusions**: Photomicrographs obtained from IHC studies revealed the presence of both an all-isoform (pan-specific) VGSC antibody (Ab) and the Ab specific for VGSC isoform $Na_v1.7$, indicating the presence of these protein channels in arterial smooth muscle cells. Western blotting with a pan-specific VGSC Ab detected the presence of VGSCs in arterial tissue.

FRITZ, JM

ALVEOLAR MACROPHAGES AUGMENT MURINE EPITHELIAL CELL GROWTH AND ATTENUATE PROSTAGLANDIN PRODUCTION. JM Fritz (Ph.D., GS), EF Redente, LD Dwyer-Nield and AM Malkinson, Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado, Denver, CO.

In both murine and human lung cancer, inflammation promotes the growth of initiated epithelial cells, while anti-inflammatory signals are potently anti-tumorigenic; e.g., over-expression of prostaglandin I_2 synthase significantly reduces lung tumor growth. Neoplastic expansion requires increasing numbers of alveolar macrophages, while macrophage ablation decreases tumor burden and number. Therefore, tumor-associated macrophages (TAM) actively enhance lung tumorigenesis. *In vitro* co-culture of an alveolar macrophage cell line (MH-S) with both non-tumorigenic and neoplastic mouse lung epithelial cell lines greatly increases epithelial cell proliferation. This growth stimulus is selective, continues with time in co-culture, and is additive to the growth-stimulating effects of serum. Perhaps mediating this effect, the pErk / total Erk ratio increased many-fold in response to the presence of MH-S cells, while the Erk/p38/Jnk phosphatase MKP-1 remained unchanged. Macrophage co-culture significantly decreases media levels of prostaglandins produced by epithelial cells; both pro-inflammatory PGE_2 and anti-inflammatory PGI_2 levels dropped nearly 50% after 48 hours. MH-S macrophages themselves produce very low levels of PGE_2 and PGI_2 (50-100 pg/mL); non-neoplastic cells produce large amounts of PGI_2 (60 ng/mL), while neoplastic cells much more PGE_2 (10-20 ng/mL). MH-S macrophages do not pinocytose or degrade media prostaglandins, and thus likely depress epithelial prostaglandin production. Consistent with this hypothesis, protein levels of the prostaglandin catabolic enzyme, 15-hydroxy prostaglandin dehydrogenase, increase. Differing patterns of sensitivity or resistance to macrophage-induced stimulation of growth and inhibition of prostaglandin production imply that these processes are not interdependent. Elucidating this mechanism of macrophage-assisted neoplastic growth may provide novel means of halting tumor progression. (Supported by USPHS Grant CA33497)

HARMEL, JL

PSYCHOSOCIAL VARIABLES AND FUNCTIONAL STATUS FOLLOWING LOWER EXTREMITY AMPUTATIONS SECONDARY TO DIABETES. JL Harmel, (MD, SOM), M Bruntz, AE Williams, J Newman, WR Smith, Department of Orthopaedics, Denver Health Medical Center, Denver, CO.

Purpose: Studies indicate that traumatic amputations deleteriously affect physical and psychosocial functioning; however, no studies have examined physical and psychosocial variables in relation to diabetic amputation. The purpose of this study was to evaluate functional and psychological outcomes of patients who had amputation secondary to diabetes. **Methods:** Patients 18 to 80 years who underwent lower extremity amputations completed the medical outcomes score short form (SF-36), and the hospital anxiety and depression scale (HADS) during a clinic visit or by mail. SF-36 scores were transformed to z-scores and compared to normative data generated from a sample of the United States population. Demographic and clinical data were collected by chart review. Univariate statistics were performed to summarize demographic and clinical data and t-tests to compare functional outcomes by psychological status. **Results:** Twenty-four patients (20 male, 4 female) participated. Thirteen (54%) reported current tobacco use. Ten (42%) had a documented psychiatric diagnosis. Comorbid conditions included: 21 (88%) cases of

hypertension, 12 (50%) cardiovascular disease, 13 (54%) history of renal failure, 9 (38%) history of retinopathy, and 17 (71%) neuropathy. Mean glycosylated hemoglobin level was 8.1%, BMI 29.6 kg/m^2, and total cholesterol 154.7 mg/dl. Mean HADS scores indicated normal levels of anxiety and depression (5.5 and 6.2, respectively). SF-36 subscale scores were below average norms for the physical function, physical role, general health, and social function subscales. Mean scores were within 1 standard deviation of norms for bodily pain, vitality, emotional role, and mental health subscales. Patients with anxiety scored significantly lower on the vitality, emotional role, and mental health subscales, and had a greater incidence of depression ($p < 0.05$). Patients with depression scored significantly lower on all subscales of the SF-36 ($p < 0.05$). Four of five patients categorized with 'moderate to severe anxiety' (HADS=11- 21), and three of six patients with 'moderate to severe depression' (HADS=11-21) had no documented psychiatric diagnosis. **Conclusion and significance:** Considering the multiple health problems affecting the study participants, it is notable that their SF-36 scores were not significantly lower than general population norms. However, scores were significantly lower for those with anxiety and/or depression. As most patients demonstrating moderate to severe levels of anxiety and depression had no documented psychiatric diagnosis, it is critical to address psychological status when caring for patients with lower extremity amputations.

HOWE, S

DENTIN BOND STRENGTH OF THIOL-ENE/METHACRYLATE COMPOSITE RESTORATIVE MATERIALS. Sara Howe,(DDS, University of Colorado Denver School of Dental Medicine), Sheldon M. Newman, DDS, MS, Biomaterials

INTRODUCTION: A novel polymer with a different photo-polymerization mechanism from that of methacrylates is being developed as the matrix for a dental composites. The new polymer offers increased strength and higher conversions. Bonding to tooth structure requires the continuous polymerization of the resin in the bonding agent with the resin matrix of the composite restorative, a process which can be compromised by the different polymerization mechanism.

OBJECTIVE: The goal of this investigation is to determine the dentin bond strength of various thiol-ene/methacrylate based composite systems with either a total etch or self etching bonding agent?

MATERIALS AND METHODS: Extracted human teeth were sectioned, dentinal surfaces polished flat to 600 grit, and mounted in a "single-plane shear test" apparatus. This surface was treated with either a total etch technique (37% phosphoric acid, followed by Optibond Solo), or a self etching technique (Clearfil S3). The 4 composite formulations bonded to the treated dentinal surface were: conventional BisGMA/HDDMA, 80:20methacrylate:thiol-ene, 70:30methacrylate:thiol-ene, and 50:50 methacrylate:thiol-ene. Each resin system was filled to 75wt% with glass. All composite and bonding layers were cured for 40 seconds at 500 mW/cm^2. The bonded teeth were stored for 2 days in a humidor at 37^{o}C. They were allowed to equilibrate to ambient room conditions before stressing the bond at a rate of 1 mm/min.

RESULTS

(mean±sd) MPa)	Conventional	80:20	70:30	50:50
Total etch	38.2±7.7	41.4±8.5	32.1±9.9	15.0±10.8
Self etch	28.6±4.7	26.3±4.7	23.5±4.5	20.8±6.2

The statistical analysis indicated that the 50:50 mixture had significantly lower bond strengths. Trends in the data suggest that the more thiol-ene, the lower the bond strength for the total etch technique. The self-etching system did not show such compromise possibly due to the potential for acid catalysis of the thiol-ene system.
CONCLUSION: Methacrylate/Thiol-ene matrix composite restoratives will bond to dentin using either conventional bonding system.

JOHNSON, SA

COUPLING OF 3'-END PROCESSING TO mRNA EXPORT. **SA Johnson, (Ph.D., GS) and D.L. Bentley, Department of Biochemistry and Molecular Genetics, University of Colorado, Denver, CO.**

In eukaryotes, expression of protein-encoding genes is a regulated, multi-step process that begins in the nucleus with transcription, and ends in the cytoplasm with translation. Transcription by RNA polymerase II (pol II) results in the formation of messenger RNA precursors (pre-mRNA). Pre-mRNAs are processed, e.g. capped, spliced, and polyadenylated, prior to their export from the nucleus. Recently, it has become clear that transcripts produced by pol II are processed co-transcriptionally, thereby "coupling" the activities of pol II to the activities of the processing machinery.

Likewise, it is becoming increasingly evident that many processing/packaging steps are interconnected with one another. An example of such a "connection" is the dependence of mRNA export on proper 3'-end formation. In budding yeast, cleavage-polyadenylation requires the action of three multi-subunit factors, including CF1A, CFIB, and CPF. These factors make multiple contacts with each other, pol II, and with the nascent mRNA. It is unknown whether any of these factors make physical contact with export factors. However, temperature sensitive (ts) yeast strains that harbor mutant components of CF1A or CPF, which disrupt cleavage and/or polyadenylation, produce transcripts that fail to exit the nucleus, and instead accumulate at the site of transcription. Reciprocally, ts mutants of some export proteins result in transcripts with improperly processed 3'-ends. Taken together, these studies strongly support the idea that 3'-end processing and export are linked. The mechanism by which this linkage occurs is unknown.

Here we demonstrate using chromatin immunoprecipitation (ChIP) that functional loss of any CF1A subunit results in failed recruitment of the essential export factor, Yra1. This lack of Yra1 crosslinking is not a function of transcript degradation. Furthermore, biochemical analyses including *in vitro* transcription/translation reactions, GST pull-downs, co-immunoprecipitation and immunoblotting indicate that CF1A, via the Pcf11 subunit, and Yra1 make physical contact. This binding requires the conserved REF domains in Yra1 and the C terminal portion of Pcf11, and does not require other proteins or nucleic acids (i.e. it is direct). Importantly, RNA tethering was used to confirm that Yra1 and Pcf11 physically interact *in vivo*. This interaction appears to be conserved in human. Using these and other data we have devised a model that integrates the mechanisms by which Yra1 is recruited to actively transcribed genes, transferred to properly processed mRNA, and facilitates mRNA export.

KAUVAR, EF

ANALYSIS OF NIPBL MISSENSE MUTATIONS IN HUMANS AND *DROSOPHILA*: IMPLICATIONS FOR THE PATHOGENESIS OF CORNELIA DE LANGE SYNDROME. EF Kauvar, (M.D., SOM), MA Deardorff, M Kaur, D Yaeger, L Jackson, D Dorsett, ID

Krantz; Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA.

Cornelia de Lange Syndrome (CdLS; OMIM 122470, 300590 and 610759) is a dominant multisystem developmental disorder characterized by facial dysmorphia, hirsutism, growth and cognitive retardation, gastroesophageal dysfunction, and genitourinary, cardiac, limb and other systemic abnormalities. In 2004, mutations in *NIPBL (Nipped-B Like)* were demonstrated to cause CdLS. NIPBL orthologs in yeast (Scc2/Mis4) and in *Drosophila* (Nipped-B) are known to be required for sister chromatid cohesion and long-range transcriptional regulation. Subsequently, mutations in other Cohesin complex members *SMC1A* and *SMC3* have been identified in CdLS. Unlike previous work in bacteria, fungi and vertebrate systems on SMC1A and SMC3, much less is understood about the function of NIPBL orthologs. Despite its large size and high primary sequence conservation between orthologs, NIPBL demonstrates little structural homology to other proteins. NIPBL is known to be involved in sister chromatid cohesion, though many of its functional domains have not been characterized. The exact mechanism by which the protein affects cohesion function is largely unknown. Furthermore, despite several screens, few mutations have been reported in yeast and *Drosophila* that serve to dissect the functional units of this protein. We have collected a cohort of over 400 probands with clinically suspected CdLS and have performed extensive mutational analysis of *NIPBL*. Mutations in *NIPBL* have been found in approximately half of CdLS cases. Of these mutations, the majority presumably leads to reduction of mRNA stability or expression, while about one quarter are missense mutations. To further clarify the domains of NIPBL essential for its function in human development (and *Drosophila*), we undertook detailed analysis of the missense mutations. Here we report 64 missense mutations in human *NIPBL*, and three missense mutations in *Drosophila Nipped-B*, the first to be reported in this organism Analysis of the distribution of these variations in NIPBL amino acid residues and correlation with human and *Drosophila* phenotypic data demonstrates 1) that multiple domains are critical for normal functioning of the NIPBL protein, 2) several domains suggest relative hot spots for mutations, and (3) conservation of phenotypic repercussions of amino acid alterations between human and *Drosophila*, all of which suggest domains that are more central to the pathogenesis of the CdLS phenotype.

KELLY, WC

IMPROVED GLYCEMIC CONTROL WITH REAL LIFE USE OF CONTINUOUS GLUCOSE MONITORING. WC Kelly, (MD candidate, SOM), SK Garg, MK Voelmle, PJ Ritchie, PA Gottlieb, KK McFann, and SL Ellis; Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Aurora, Colorado

Purpose of Study: Improving glycemic control reduces micro- and macro-vascular complications associated with diabetes. It is not known if real life use of Continuous Home Monitoring of Glucose (CHMG) can improve glucose control. This study evaluates changes in glycemic control that are observed in real life use of real time CHMG.

Methods Used: Forty Seven adult subjects with type 1 diabetes were identified for this analysis. Twenty four subjects had CHMG data at 1, 6, and 12 weeks as well as A1c values at baseline and 3 months. These subjects were then computer matched to 23 controls for age, gender, duration of diabetes, and baseline A1c values. There were no significant differences in baseline characteristics between comparison and CHMG groups. Before using CHMG subjects attended a class that covered glucose trends, features of the CHMG

receiver, insertion techniques, and the importance of confirming glucose values with self monitored blood glucose.
Summary of Results: Each sensor was used for 6.8 ± 1.6 days (mean ± SD), despite approval for 3 days, and each subject used their sensor a mean of 17.6 ± 8.4 days per month. There was a significant reduction in A1c values (0.4 ± 0.5%; p<0.001) in the CHMG group at 12 weeks without any change in insulin dose, whereas the control group showed a non significant increase in mean A1c values (0.3 ± 1.1%; p=0.178). Also, at 12 weeks there was a difference in A1c between groups (p = 0.0385). Mixed model repeated measures analysis showed an increase in mean percent of glucose readings within the target range (60-150 mg/dL) and a decrease in glucose readings above the target range (> 150 mg/dL) at 12 weeks when compared to baseline (p=0.035). This significant improvement in glucose readings was observed without an increase in mean percent of glucose readings below the target range (< 60 mg/dL). In addition the number of subjects achieving A1c values < 7.5% was higher in the CHMG group at 12 weeks (OR = 7.229, p = 0.0234).
Conclusion: These results show that relatively well controlled subjects with type 1 diabetes can further improve their glucose control with CHMG. This is the first real life study which demonstrates that clinical use of CHMG improves A1c values, with a significant increase in target range glycemia, without increasing hypoglycemia.

KERR, CA

EXPRESSION OF PEA3 MAY CONTRIBUTE TO THE MALIGNANT PHENOTYPE OF COLON CANCER CELLS *IN VITRO*. CA Kerr, Jedlicka P (MD, PhD), Gutierrez-Hartman A (PhD), and Yaghi NK, Department of Endocrinology, University of Colorado, Aurora, CO.

Pea3/E1AF/Etv4, a member of the PEA3subfamily of Ets transcription factors, is overexpressed in a number of malignancies. In colon cancer, its overexpression is an independent predictor for poorer prognosis, likely in part due to its regulation of carcinoma invasive behavior. Members of the Pea3 subfamily synergize with the ß-catenin-LEF-1 complex to activate transcription of Matrilysin, and this likely represents an important mechanism by which Pea3 promotes tumor invasion. However, the full mechanistic spectrum of Pea3 tumor-promoting effects is not known. Here, we show that expression of Pea3 protein is directly correlated to the biological aggressiveness of colon cancer cells *in vitro*, and attempt to demonstrate that reducing endogenous Pea3 levels will lead to a less malignant phenotype. Using lentiviral infection, we introduced five distinct small interfering RNAs into Pea3 over-expressing colon cancer cells (HT29 and SW620), and measured the effects on cell proliferation, migration and invasion. Preliminary results show good correlation between endogenous levels of Pea3 expression and invasion and migration in three colon cancer cell lines of varying biologic aggressiveness (CaCo-2, HT29, and SW620). ShRNA expression potently reduced the Pea3 protein levels in HT29 and SW620 cells, and resulted in some, although variable, reduction in cell motility, without significantly affecting cell proliferation. These initial results are encouraging, but further studies will be necessary to fully elucidate the tumor-modifying effects of Pea3 in colon cancer.

KLEINMAN, B

VARYING HYDROPHILIC RESIN COMPOSITE MATRICES AFFECTS ON STRENGTH VERSUS TIME

Brian Kleinman DDS candidate in The School of Dentistry, Sheldon M. Newman, DDS, MS, Biomaterials

INTRODUCTION- Composite resin matrices can be made from varying dimethacrylates with different potentials to absorb water. A new comonomer system using dimer-acid dimethacrylate (DAD) polymerizes into a phase separating structure which could have unique water sorption potential. Water sorption can affect flexural strength and flexural modulus over time. Also the composite continues to have a slow increase in conversion which can increase strength and modulus.

OBJECTIVE- The goal of this investigation is to determine the effects of water sorption and continued conversion on flexural strength over months by storage in both wet and dry conditions.

MATERIALS AND METHODS- Composites were formulated with the following comonomer systems: BisGMA/TEGDMA, UDMA/TEGDMA, EthoxyBisGMA/HDDMA, BisGMA/HDDMA, and UDMA/EthoxyBisGMA/DAD. Each resin was filled 75wt% with conventional glass filler. Flexural strength beams 2mmX2mmX25mm were prepared, and conversion determined initially with FTIR spectra. The specimens were stored in either water or dry at 37°C, for 24 hours, 48 hours, and 1 month. At time of testing all specimens were allowed to equilibrate to ambient room conditions, the conversion determined again and the beams tested by 3-point bending.

RESULTS -The highest initial strength was observed in the dimer-acid dimethacrylate composite. By 2 days the wet storage conditions produced lower flexural strengths than the dry storage for the same composite formulation. The differences increased by one month. The flexural modulus remained constant or increased when stored dry. The wet storage conditions decreased the modulus in most composite formulations.

CONCLUSION- Generally the wet storage conditions caused a lower flexural modulus and flexural strength than that produced by dry storage conditions for the same composite stored dry. The highest initial strength was exhibited by the dimer-acid based composite.

KOVACS, JM

DEFINING CD21-LIGAND INTERACTION USING LIGAND SPECIFIC INHIBITORY PEPTIDES

JM Kovacs (Ph.D., GS), JP Hannan, VM Holers: University of Colorado Denver - School of Medicine, Departments Medicine/Immunology, Denver, CO 80045

Complement receptor 2 (CR2, CD21) is a cell membrane protein, with 15 or 16 extracellular short consensus repeats (SCRs), that promotes B cell immune responses. The most distally located SCRs (SCR1-2) mediate the interaction of CD21 with its four known ligands (C3d, Epstein Barr Virus (EBV) gp350, Interferon (IFN)-alpha, CD23). Anti-CD21 inhibitory monoclonal antibodies (mAbs) block binding of all ligands equivalently. Ligation of CD21 to three of its ligands are natural and required for normal B cell immune responses; however, ligation of the fourth ligand, gp350, allows for infection by EBV, a pathogen that is known to cause many life-threatening conditions. To develop ligand-specific inhibitors that would also assist in identifying residues unique to each receptor-ligand interaction, phage were selected by panning with SCR1-2 from constrained and un-constrained libraries, followed by ligand-driven elution. Derived peptides were tested by competition ELISA. Peptides identified against C3d and gp350 showed low and mid micromolar ligand-selective inhibition, respectively. The peptide blocking C3d, as well as full-length C3d, were titrated into N-15 labeled SCR1-2, which revealed specific nuclear magnetic resonance (NMR) chemical shift changes indicative of specific CD21 side-chain

interactions. These peptides represent the first method of selectively inhibiting single ligands of CD21 and have the potential to determine the effect of inhibiting a single ligand-CD21 interaction during a complex immune response. Inhibitory peptides against gp350 represent a possible therapeutic against EBV. Funded by R01 CA053615 awarded to VMH from the NIH.

LANGE, CM

PERCEPTIONS OF APPROPRIATE USE OF OVER THE COUNTER PAIN MEDICATIONS IN EMERGENCY DEPARTMENT PATIENTS. CM Lange (MD, SOM), C Gogela, K Heard, Department of Emergency Medicine, University of Colorado, Denver, CO.

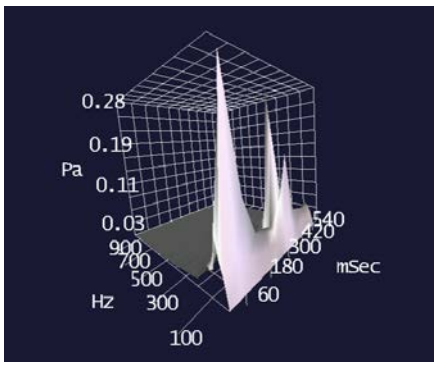
Background: Over the counter pain medications (OTCPM) are widely used by ED patients. Inappropriate use of these medications can result either in undertreatment or toxicity. *Objective:* The objective of this study was to determine what proportion of ED patients taking OTCPM had contraindications to the medications and what proportion knew the maximum daily dose of their OTCPM. *Method:* We conducted a survey of ED patients using a random time block design. Patients were asked what OTCPM they were taking, in what dose, and for how long. Patients were then asked to state what they believed the maximum daily dose was for each medication they were taking, and how they chose the dose that they were using. Patients were asked about contraindications to OTCPM (more than 3 alcoholic drinks per day, kidney problems, gastric ulcers or the use of daily aspirin for cardioprotection). We also identified patients taking multiple OTCPM. Proportions with 95% confidence interval were determined. *Results:* A total of 599 ED patients were included; 120 patients refused or could not be consented. Interviewed subjects (n=479) reported the following use: acetaminophen 114 (24%), ibuprofen 99 (21%), aspirin 27 (6%) and naproxen 8 (2%). 44% (35 to 54) of NSAID users reported having at least one contraindication. Only 1 patient taking APAP reported having a contraindication (consuming greater than 3 alcoholic drinks per day). 22% of patients reported OTCPM use longer than 10 days, the maximum recommended duration. 51% (45 to 58%) of patients stated that they knew the maximum daily dose of their medication. 32 of the 162 estimated maximum doses made by patients were in excess of the maximum recommended dose for that medication. *Discussion:* ED patients frequently use OTCPM but have insufficient information about the proper dosing and contraindications. Results may be limited by reporting bias and by refusal of 20% of patients.

LATERZA, R

APPARATUS AND METHODS FOR ANALYZING HEART SOUNDS Ryan Laterza, (M.D., SOM), Howard Weinberger, University of Colorado Denver School of Medicine, University of Colorado at Boulder College of Engineering, National Jewish Medical and Research Center

This biomedical device is intended to improve the methods and diagnostic capabilities of medical professionals in diagnosing heart murmurs. Physicians currently utilize a traditional, passive acoustic stethoscope and their perception of heart sounds for their initial diagnosis. This biomedical device generates a three-dimensional plot showing quantified measured data of recorded heart sounds which is inherently more definitive and accurate than traditional stethoscopes permit.

This device appears and functions similar to the traditional stethoscope allowing the physician to listen to heart sounds while in use with the exception of a small circuit that records the ECG and heart sound data onto a small memory card. This memory card is then removed from the stethoscope and connected to a computer for the Fourier transform computation of the heart sounds using ECG R waves as a reference to generate a three-dimensional time-varying frequency-magnitude spectrum plot.



Aortic Valve Recording Demonstrating S2 Splitting

LIEN, TM

A COLLABORATIVE CARE MODEL: A DESCRIPTIVE STUDY OF CO-LOCATED MENTAL HEALTH AND PRIMARY CARE PROVIDERS. TM Lien, (M.D., MS), A Talmi, and B Stafford, Psychiatry Scholars Program, Department of Psychiatry & Behavioral Sciences, University of Colorado, Denver, CO.

Background. Three-quarters of children with psychiatric disorders and manifestations are seen in primary care settings, and 15-20% of children and adolescents in pediatric primary care have a behavioral health disorder. The co-location of mental health care providers with primary care pediatricians enhances collaboration in the assessment and treatment of pediatric behavioral disorders. Goals of collaboration include: facilitating earlier detection of mental illness in children, improving patient access to pediatric mental health treatment, and improving the ability of primary care physicians to make diagnoses and initiate treatment.

Objectives. The objectives were (1) to develop a method of evaluating collaborative care services, (2) to identify the levels of collaboration between primary care providers and mental health care providers in a pediatric clinic, (3) to describe the characteristics of collaboration between these providers, and (4) to relate collaborative care to quality of care and health systems outcomes.

Methods. Patient records of 77 children who were under the age of 4 at the time of initial mental health consultation and were seen at the Child Health Clinic, located at The Children's Hospital, were reviewed. Descriptive measures of collaboration, as well as individual and health systems outcomes were abstracted. Traditional levels of collaborative care were coded based on services received.

Results. Of the 77 children and their caregivers that received collaborative services for mental health/behavioral or developmental concerns, 7 (9.1%) received parallel delivery of services, 2 (2.3%) received informal consultations, 38 (49.4 %) received formal consultations, 11 (14.3%) had co-provision of care by providers, and 19 (24.7%) had collaborative networking between professionals regarding their care. Data analyses

(ANOVAs) examined individual and health systems outcomes for each of the traditional levels of collaboration. Results indicated that with more intensive collaboration there was an increased likelihood of a new diagnosis and a greater tendency for receiving mental health or behavioral services. Results did not show a relationship between levels of collaboration and having up-to-date well child checks and immunizations.
Conclusions. A theoretically derived coding system for determining the extent of collaborative care was developed and used to determine levels of collaboration between primary care providers and mental health consultants. Levels of collaboration were related to individual and health systems outcomes.

LINNELL, E

DERMATOLOGY FACULTY EVALUATION BY RESIDENTS. E. Linnell, (MD, SOM), C. Nelson, MD, S. Freeman, MD, D. Crockett, L Umstattd, R.P. Dellavalle, MD, PhD, MSPH. University of Colorado School of Medicine, Department of Dermatology, Denver, CO

Purpose: To describe resident evaluation of dermatology faculty performance.

Methods: The program directors of 112 ACGME accredited dermatology residency programs were mailed a twenty-item survey, and asked to return a blank copy of any evaluation form(s) used to evaluate faculty at their program.

Results: After two mailings, 87% returned the completed survey (97/112) and 56% (54/112) returned copies of the faculty evaluation forms used. Two programs reported that residents do not evaluate the dermatology faculty. Four programs reported using different forms to evaluate faculty in different dermatology subspecialties (e.g. dermatopathology vs. pediatric dermatology). Ninety-two percent of programs have residents complete the forms anonymously. Fifteen percent of respondents said that faculty evaluations by residents are not important for faculty promotion; 67% somewhat important; 18% very important; and 20% did not to respond to this question. The distribution of the evaluations to the residents is most often a paper form distributed to resident mailboxes (33%) followed by electronic forms sent to resident email accounts (29%). The most common components of the evaluations were asking for other comments (50/54) and rating faculty teaching (50/54) and availability/accessibility (40/54).

Conclusions: Residents commonly evaluate dermatology faculty teaching and knowledge anonymously without regard to faculty subspecialization. Because these evaluation forms may affect faculty promotion, we suggest using evaluation forms that account for dermatology field subspecialization to provide more accurate evaluation of faculty teaching.

MCCOY, EM

IN VIVO ROLE OF THE SIX1 HOMEOPROTEIN IN MAMMARY GLAND TUMORIGENESIS. EM McCoy (Ph.D., GS), A Welm, K Heichman, P Jedlicka, L Chodosh, HL Ford. Program in Molecular Biology, University of Colorado Health Sciences Center, Aurora, Colorado.

Human Six1 is a homeodomain-containing transcription factor that is critical for cell proliferation, survival, and epithelial-to-mesenchymal transition (EMT) during normal development. In addition to its developmental role, overxpression of Six1 has been detected in a number of human cancers, including breast cancer, where it is linked to both proliferation and metastasis. As many as 50% of primary breast cancers and 90% of metastatic lesions overexpress the gene, in part due to gene amplification. Six1 can

transform a mammary epithelial cell line, but no work has been done to show the effects of Six1 overexpression in vivo. We have established an inducible, mammary-specific Six1 overexpression model by crossing MMTV-rtTA mice to TetO-Six1 mice, and are using this model to test whether Six1 overexpression leads to mammary tumors, as well as to dissect the molecular mechanism by which Six1 influences tumorigenesis in vivo. Low levels of Six1 expression is observed in uninduced bitransgenic animals over long periods of time, suggesting leakiness in the inducible model. Interestingly, animals treated with doxycycline, as well as uninduced animals, develop marked mammary hyperproliferation and abnormal alveologenesis. In addition, tumor formation is observed after long latency (>1 year) in both induced and uninduced animals, suggesting that low levels of Six1 are sufficient to cause transformation in this model. Tumors formed are complex, but are best characterized as invasive ductal adenocarcinomas with complex features. Importantly, sarcomatoid differentiation (spindle cell morphology) is observed, and E-cadherin expression is lost, while the mesenchymal markers Zeb1 and □-catenin are detected in the nuclei of spindle-cell areas of the tumors. Nuclear localization of □-catenin in tumors overexpressing Six1 suggests that the Wnt pathway, a potent mediator of tumorigenesis, may be activated in Six1-driven tumors. Finally, lung metastasis has occurred in a subset of animals. Thus, this transgenic model demonstrates that inappropriate expression of Six1 promotes high-grade tumor formation, oncogenic EMT, and metastasis, suggesting that Six1 is a powerful oncogene that is important not only for tumor initiation, but also for tumor progression. Mining of clinical data sets reveals that expression of the Six1 transcriptional complex is an indicator of poor prognosis in a number of different cancers, suggesting that Six1 may play important roles in many different cancer types. As Six1 is not necessary for most normal adult tissues, therapies directed against Six1 may not lead to the severe side effects seen with more conventional treatments, making Six1 an attractive chemotherapuetic target.

MCKEAN, DM

THE ROLE OF THE GPI-ANCHOR IN TGF□ SIGNALING AND FOREBRAIN DEVELOPMENT. DM McKean (Ph.D., GS) and L Niswander, Department of Pediatrics, University of Colorado, Denver, CO.

Purpose: In humans, holoprosencephaly (HPE) occurs in 1 out of 250 pregnancies, usually resulting in spontaneous abortion. HPE is a defect of the developing forebrain, which is critical for higher thinking, as well as for sensory and motor functions. I have identified Phosphatidylinositol Glycan, class N (Pig-N) as a novel gene that when mutated leads to HPE in mice. Pig-N is involved in synthesizing glycerophosphatidyl inositol (GPI) anchors that are then covalently attached to a diverse group of target proteins. These GPI anchored proteins (GPI-APs) are targeted to and localized in lipid raft domains of the plasma membrane. I have hypothesized that the Pig-N mutation results in HPE due to abnormal processing and activity of Cripto, a GPI-AP that is involved in forebrain specification. To test my hypothesis I will (1) verify that the mutation in Pig-N is responsible for the HPE phenotype, and (2) demonstrate that GPI-APs are mislocalized, and (3) show that Cripto activity is disrupted in Pig-N mutants.

Methods: To verify that the Pig-N mutation is responsible for the HPE phenotype I have made and am characterizing a BAC transgenic mouse containing a wildtype copy of the Pig-N gene. I will see if this BAC can rescue the HPE phenotype in homozygous Pig-N -/- embryos. To demonstrate that GPI-APs are mislocalized in Pig-N -/- embryos, I will cross

the gonzo mouse line into a GPI-GFP mouse line and look at GFP localization. Furthermore, I will look at Cripto localization by immunohistochemistry. To look at Cripto activity, I will perform luciferase assays using a Cripto-responsive promoter in cells derived from Pig-N mutant embryos versus wildtype cells. Furthermore, I will look at Cripto activity in vivo, using a Nodal-LacZ reporter mouse.

Results: GPI-GFP is mislocalized in Pig-N mutant embryos, as well as in cell lines derived from those embryos. Cripto activity is altered in these cell lines as well; surprisingly, Cripto activity appears to be upregulated in Pig-N -/- cells.

Conclusion: While I have not proven that the mutation in Pig-N is responsible for the HPE phenotype, there are two lines of evidence that suggest that it is: it is the only mutation that I found while sequencing, and another GPI biosynthesis gene mutation results in HPE. It appears that the altered processing of the downstream target Cripto, is responsible for HPE in the gonzo line.

MICALIZZI, DS

THE ROLE OF TRANSFORMING GROWTH FACTOR-BETA SIGNALING IN SIX1-INDUCED EPITHELIAL-TO-MESENCHYMAL TRANSITION AND METASTASIS. DS Micalizzi, (M.D./Ph.D., GS), KL Christensen, C Aldridge, HL Ford, Molecular Biology Program, University of Colorado Health Sciences, Aurora, CO.

During embryogenesis, the homeodomain transcription factor Six1 is critical in the development of multiple organs where it directs precursor cell proliferation, cell survival and epithelial-to-mesenchymal transitions (EMT). In most normal adult tissues including the mammary gland, Six1 is not expressed, however, in human breast cancer it is re-expressed in 50% of primary and 90% of metastatic lesions and significantly correlates with shortened time to metastasis and to relapse, and with decreased disease-specific survival. Together, these data suggest that Six1 may contribute to tumor progression. The expression of Six1 in both the immortalized mammary epithelia cell line, MCF12A, and the mammary carcinoma cell line, MCF7, increases expression of Transforming Growth Factor-Beta Receptor I (T□RI). Increased expression of T□RI in mammary cells correlates with increased TGF-□ signaling as measured by phosphorylation and nuclear localization of Smad3 and increased basal and TGF-□-induced expression of the TGF-□-responsive reporter, 3TP-luciferase. Activation of the TGF-□ pathway in response to Six1 begs the question whether TGF-□ signaling underlies Six1's role in tumor progression. TGF-□ is a known inducer of EMT, a process that is correlated with increasing malignant potential in cancer. Indeed, Six1 expression in mammary cells induces properties of EMT including downregulation or relocalization of E-cadherin, increased □-catenin transcriptional activity, alterations in cell-matrix adhesion, and increased invasion. More importantly, Six1 expression in the non-metastatic MCF7 cell line induces lymphatic and bone metastases after orthotopic injection into the mammary glands of nude mice, consistent with TGF-□'s pro-metastatic role in other models of breast cancer. Based on the established role of TGF-□ in EMT and metastasis particularly to the bone, we hypothesize that Six1 contributes to increased tumor malignancy and metastasis through upregulation of TGF-□ signaling.

MILLER, MP & MILLER, HJ

DEVELOPMENT OF A HEALTH NEEDS ASSESSMENT IN DENVER'S REFUGEE POPULATION. MP Miller (BA), HJ Miller (MPH), EM Aagaard (MD), RH Miranda (MD), Department of Medicine, University of Colorado, Denver, CO.

Purpose: Development of a comprehensive needs and resource assessment is essential for implementing a sustainable and effective health intervention. We developed an observational, cross-sectional study that will describe the current situation surrounding health and health care for the local refugee communities in Denver. The results of this needs assessment will be used to implement a service learning experience for preclinical medical students that meets the target populations' identified needs.

Objectives: Evaluate the self-perceived baseline health status of the refugee population in Denver; Evaluate the quality of existing services in regards to patient satisfaction, meeting population specific needs and cultural competence; Define and identify perceived financial and physical barriers in access to quality care and services for the refugee population in Denver;

Methods: The development of this assessment was carried out in 5 steps. Step 1 was choosing a model and building the context of our greater goals and population into that model. An ecological model was used. Step 2 was to outline the objectives of the assessment based on the organization that the model provided. Step 3 was to choose appropriate tools to address each objective. The study consists of questionnaires, semi-structured interviews and focus groups with refugees, key informants from the community and service providers. Step 4 was the development of these tools. This step included: a literature search for relevant and validated tools, creation of new tools, editing and piloting of developed tools. Step 5 consisted of protocol development and implementation strategies, both of which flow from the organization created in steps 1 and 2.

Results: This process has created a cross-sectional study that is built around the ecological model of health to assess the following influences on refugee health and well-being in Denver: intrapersonal, interpersonal, institutional, community and environmental. The Assessment will target three populations with active interests in refugee health: refugees at three stages of arrival in the USA (less than 3 months, 3 – 8 months and more than 8 months.), providers of direct and ancillary services and key informants or community stakeholders. The study will implement five instruments: a language-concordant questionnaire for refugees; semi-structured interviews for refugees, providers and key informants, respectively; and a series of focus groups with refugees.

Conclusion: We have developed a comprehensive needs and resource assessment of the refugee population in Denver via thorough knowledge of and collaboration with all relevant parties, and creation of appropriate methods for development and validation of instruments. The study is currently being implemented in Denver by University of Colorado Medical Students. Results are expected to be available by the spring of 2009 with a plan to develop a service learning project for medical students based on these results by 2010.

NOBLE, S

P-BODY/STRESS GRANULE-LIKE PARTICLES ARE REGULATED AND LINKED TO MATERNAL MRNA CONTROL DURING C. ELEGANS DEVELOPMENT.

Scott Noble, Brittany Allen, Lai Kwan Goh, Kristen Nordick, and Thomas C. Evans.

P bodies (PBs) and stress granules (SGs) have been proposed as sites of mRNA regulation but their functions and control in vivo are poorly understood. In C. elegans, precise control of mRNA translation is critical for early development. We find that PB/SG-like granules form during germ cell development but that these granules are diverse, are regulated by developmental signals, and have functions in mRNA control that change during development. During oogenesis and embryogenesis, distinct RNA-binding proteins

repress specific mRNAs at different stages. For Notch/glp-1 mRNA, GLD-1 and PUF-5/6/7 repress glp-1 mRNA translation through distinct 3' UTR elements. Interestingly, these proteins co-locallized with glp-1 mRNA in large granules that also contain the PB/SG factors CGH-1 (RCK/Dhh1) and CAR-1 (RAP55/Trailerhitch). Other repressed mRNAs were also targeted to these granules, but translationally active mRNAs were not. These observations suggest that specific RNA binding proteins target mRNAs to PB/SG-like mRNP granules, and that these granules participate in mRNA repression. In support of this idea, glp-1 3' UTR elements could target injected reporter mRNA to these granules. Furthermore, loss of CAR-1 and CGH-1 caused activation of glp-1 translation and disrupted granule morphology and granule targeting of mRNAs. Finally, car-1 showed potent genetic synergism with puf-5 in the control of oocyte formation suggesting that they act together to control many mRNAs. However CAR-1 and CGH-1 were only required for glp-1 repression during late stages of oogenesis. Further, CAR-1 and CGH-1 loss altered oocyte mRNP granule morphology in distinct ways suggesting they have distinct functions. The size, organization, and composition of these granules were regulated by sperm signals, fertilization, and early polarity cues. Collectively, these findings suggest that C. elegans mRNP granules are diverse in structure and function, and that they are dynamically controlled by developmental pathways. This work was supported by grants from NSF (0345386, 0725416) and NIH (RO1 GM079682).

NORDICK, K

INSIGHT INTO THE PATHOGENESIS OF RHEUMATOID ARTHRITIS FROM A CITRULLINE SPECIFIC MONOCLONAL B CELL MOUSE. K Nordick, (Ph.D., GS), CL Cozine, MG Velez, R Pelanda, VM Holers. University of Colorado at Denver and Health Sciences Center

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes joint inflammation, leading to severe pain and loss of movement. Both genetic as well as environmental factors have been implicated in the pathogenesis of RA. The inherited human leukocyte antigen (HLA) allele, DR4, is associated with genetic susceptibility for RA. Numerous environmental factors have been linked to RA pathogenesis, and we hypothesize that these environmental factors trigger an inflammatory event causing proteins to become citrullinated at arginine residues. The presence of citrullinated proteins in the joints of RA patients correlates with disease severity, and RA patients also uniquely produce high levels of anti-citrullinated protein antibodies (ACPAs). Interestingly, peptides from citrullinated protein antigens expressed in inflamed joints of RA patients may fit particularly well into HLA DR4 molecules. Furthermore, ACPAs enhance tissue injury in animal models of autoimmune arthritis, are present before RA disease is evident, and are highly specific for RA.

The purpose of this study is to determine if a monoclonal B cell mouse that reacts specifically with citrullinated epitopes can give us insight into loss of tolerance mechanisms that we believe are associated with this autoimmune disease. This mouse would help us understand the contribution of citrullinated epitopes to the pathogenesis of an autoimmune disease by letting us track specific B cells through their development. In addition, these mice can be used to look at processes of B cell development that normally occur, such as somatic hypermutation and class switch recombination.

This mouse will be constructed by the use of a hybridoma, designated D5, which produces IgM antibody that specifically recognizes citrullinated epitopes. The variable domains from the D5 heavy (H) and light (L) chains will be homologously recombined into the Ig loci of an

embryonic stem (ES) cell line. ES cells that are positive for the D5 insert will be selected, injected into the blastocysts of an embryo, and implanted into pseudopregnant female mice. Transgenic pups will be screened for the presence of B cells with the D5 H and L chains. We will then determine if B cells expressing the knocked-in D5 Ig genes develop and if they are anergized. B cell development and lineage will also be evaluated, along with disease incidence, joint histology, and antigen specific responses after immunization with an arthrogen.

O'BRIEN, JH

PRO-INFLAMMATORY ROLE OF TUMOR MICROENVIRONMENT IN PROMOTING METASTASIS IN PREGNANCY-ASSOCIATED BREAST CANCER. JH O'Brien (Ph.D., GS), TR Lyons, S Wilson, KR Shroyer, SM Lucia,V Borges, and P Schedin. Department of Medical Oncology, University of Colorado, Denver, CO.

Our research goals include characterizing the pro-inflammatory component of mammary gland regression (involution) and determining the potential role this environment plays in promoting tumor metastasis in pregnancy-associated breast cancer (PABC). Full term pregnancy in older women increases breast cancer risk for at least five years after pregnancy and breast cancer diagnosed during this time has been referred to as PABC. Importantly, PABC patients have worse prognosis than age-matched women who have not had a recent pregnancy. Epidemiologic studies find that the poorer survival associated with PABC is independent of known prognostic factors. We hypothesize that the tissue microenvironment of the post-pregnant gland is tumor promotional, utilizing several mechanisms of the immune system to promote tumor cell migration, invasion, and metastasis. Following parturition or lactation, in a process called involution, the breast returns to its pre-pregnant state. Using the Sprague Dawley rat model, we have found that mammary involution employs some of the same tissue remodeling programs activated during wound healing and inflammation including high matrix metalloproteinase activity, release of bioactive fragments of fibronectin and laminin, deposition of fibrillar collagen, and increased cytokine levels. This physiologically normal, but pro-inflammatory remodeling of the mammary gland may account for the high rate of metastases seen in PABC. Consistent with this hypothesis, our lab found that extracellular matrix (ECM) isolated from mammary glands of rats undergoing involution promoted tumor cell invasiveness in cell culture and in xenograft models of metastasis more than mammary ECM isolated from virgin rats. To define involution features common to inflammation, the question of whether immune cells are recruited to the involuting gland was investigated in both rat mammary and human breast tissue. In the rat model, putative macrophage number, as measured by CD68, was immunohistochemically analyzed in panel of mammary gland stages. The number of CD68 positive cells/mm^2 was increased up to 6 fold during involution compared to all other stages. CD68 staining in human breast tissue follows the same trend. Further characterization of the macrophages present during rat mammary gland involution revealed that the CD68 positive cells are not primarily subtype M1 (iNOS) or M2 (Arginase-1) macrophages. In our rat model, we are currently addressing whether ibuprofen or macrophage inhibitor treatment during mammary gland involution will produce changes in immune cell influx and ECM protein composition. We predict that treatment with anti-inflammatory agents during this window of gland remodeling will alter

the function of mammary gland stroma resulting in a microenvironment that is less supportive of tumor cell survival, growth, and invasiveness.

OLIVERIA, SF

LOCALIZED CALCINEURIN CONFERS Ca^{2+}-DEPENDENT INACTIVATION UPON L-TYPE Ca^{2+} CHANNELS. SF Oliveria, (M.D., Ph.D.; MS & GS), ML Dell'Acqua and WA Sather. Dept. of Pharmacology and Program in Neuroscience. University of Colorado Denver, Aurora, CO.

Ca^{2+} entry through L-type Ca^{2+} channels controls such fundamental activities of excitable cells as muscle contraction, hormone secretion and neuronal gene expression, with cellular control of L-type Ca^{2+} channel activity provided via the second messengers Ca^{2+} and cAMP. $Ca_V1.2$ L-channel opening probability is limited by Ca^{2+}-dependent inactivation (CDI) and enhanced by phosphorylation of channels via the cAMP-dependent kinase (PKA). PKA is localized to the channel by A-kinase anchoring proteins (AKAPs) like AKAP79, which targets PKA and the Ca^{2+}/calmodulin (CaM)-regulated phosphatase calcineurin (CaN, PP2B) to $Ca_V1.2$ where these enzymes antagonistically regulate channel activity in response to cAMP and Ca^{2+}. CDI has been attributed to binding of Ca^{2+}/CaM to an IQ motif in $Ca_V1.2$, a molecular mechanism distinct from phosphorylation-dependent channel enhancement. Here we show that Ca^{2+}/CaM-stimulated CaN phosphatase activity triggers CDI, suggesting that CDI, like enhancement, proceeds through changes in channel phosphorylation. Although neither the IQ motif nor AKAP79 is strictly required for CDI, both profoundly influence this process. Thus, we have found that the IQ motif and AKAP79 act in concert to maintain CaM-CaN signaling during heightened channel activity. As Ca^{2+}, CaM and CaN regulate the channel within milliseconds of Ca^{2+} influx, our findings reveal the high efficiency that can be achieved by localized signaling molecules and resolve apparent discrepancies between early reports that CDI involved rapid phosphorylation signaling and recent studies that have focused on a CaM-dependent mechanism.

ONOFREI, LV

MILD COGNITIVE IMPAIRMENT AND PET: AN EVIDENCE-BASED APPROACH TO A CASE STUDY. L.V. Onofrei (MD, SOM), R.Wetherington, MA[2],P.C. Heyn, PhD[1], A. Sage-El, MD[1], [1] School of Medicine ● University of Colorado Health Sciences Center [2] Department of Psychology ● University of Colorado at Colorado Springs.

Alzheimer's disease (AD) is the most common form of dementia and has been associated with diabetes and metabolic syndrome (MS). It is important to detect AD at early stages to allow prompt initiation of therapy and counseling of the patients and family.

Aim: The aim of the current study is twofold:1) to systematically analyze the literature to investigate the clinical significance of FDG-PET in the early diagnosis of mild cognitive impairment (MCI), and 2) to examine by a case study design the patterns of hypometabolism found in two older female subjects with metabolic syndrome and subjective memory complaints. Systematic Review Methods: An extensive review of literature has been conducted, using PubMed as the primary search engine. A set of predefined inclusion criteria was used to determine inclusion and analysis of the papers. Case Study Methods: Two older female patients (PA& PB) were evaluated by a comprehensive battery of neurological, memory, and laboratory tests. Review Results: A total of 6 studies were included in the review. Decline from MCI to AD was predicted with sensitivities ranging from 38% to 93%, specificities ranging from 62.5% to 97% and accuracies from 81.8 to 90%

(N=6). Case Results: Both patients had insulin resistance and MS. The neuropsychological evaluation indicated that both patients suffer from mild MCI. Conclusions: The results of the review of literature show that due to the high values for specificity, sensitivity, and accuracy, as well as due to the consistent patterns of hypometabolism detected, FDG-PET is a good diagnostic tool and shows great promise as a clinical tool in the early detection of MCI. As for the case study, the FDG-PET scans of PA and PB showed different patterns of hypometabolism, suggesting the patients have two different types of MCI. Even though most of the neuropsychological tests were close to normal ranges, the PET scan was able to detect patterns of hypometabolism—and thus reduced function, consistent with the subjective memory complaints of the patients. The objective assessment performed with FDG-PET was more reliable in predicting early MCI than the neuropsychological tests, as suggested by previous studies.

PARKER, T

EFFECTS OF NICOTINE ON AUDITORY GATING IN MANIC ILLNESSES. T Parker, (M.D., SOM), L Martin, J Lins, R Freedman, A Olincy, Department of Psychiatry, University of Colorado, Denver, CO.

Deficits in the ability to inhibit evoked EEG responses to repetitive auditory stimuli are noted in a variety of mental illnesses. This gating deficit is most markedly noticeable in schizophrenia, however those with a history of mania are also commonly affected. Nicotine has been shown to normalize gating ability in schizophrenia, however its effects in manic illnesses is not as commonly analyzed. This study is being undertaken to provide further data to explore potential underlying mechanisms and routes of treatment in manic illness.

Men and women between the ages of 18 and 60, both smokers and nonsmokers, were recruited from the Denver metro area. Diagnosis of either Bipolar Disorder, Type I, with or without psychosis, or Schizoaffective Disorder, Bipolar Type, was confirmed through the Structured Clinical Interview for DSM-IV Diagnoses (SCID) and a detailed life history. Three standardized surveys assessed current symptoms: Beck Depression Inventory, Young Mania Rating Scale, and the Brief Psychiatric Rating Scale. Baseline EEG recordings of the P50 auditory evoked potential were collected. Subjects then chewed either nicotine or placebo gum, and immediately thereafter P50 EEG recordings post drug administration were obtained. Subjects returned at a later date to be tested with the alternate gum, in a session otherwise identical to the first.

It is anticipated that subjects who show a reduced ability to gate P50 responses at baseline will demonstrate a marked improvement in these responses after nicotine administration, over any gain from placebo. Other studies have indicated that increased adrenergic activity causes P50 gating deficits as well. We therefore also predict that nicotine-induced gating improvements will be more significant for those with a greater degree of psychosis history. Specifically, bipolar subjects with psychosis are expected to show greater improvements than those without, and schizoaffective subjects are expected to demonstrate the most significant normalization.

Nicotine exposure leading to improvements of deficient P50 gating ability would lend evidence to the theory of a shared cholinergic mechanism. Degree of improvement correlating to degree of mania/psychosis would support the involvement of noradrenergic inhibition of interneurons as a secondary etiological pathway in P50 gating deficiencies.

PENA, PV

MOLECULAR MECHANISM OF HISTONE H3K4ME3 RECOGNITION BY PLANT HOMEODOMAIN OF ING1 AND THE EFFECTS OF CANCER SPECIFIC MUTATIONS

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Inhibitor of growth 1 (ING1) is implicated in oncogenesis, DNA damage repair and apoptosis. Mutations within the *ING1* gene and altered expression levels of ING1 are found in multiple human cancers. Despite the physiological importance of this tumor suppressor, little is known about how it functions at the molecular level. Here we report a 2.1 Å resolution crystal structure of the ING1 plant homeodomain (PHD) finger in complex with a peptide of histone H3 trimethylated at lysine 4 (H3K4Me3), and characterize the molecular mechanism of the histone recognition. We show that cancer mutations map to the region surrounding the histone peptide binding site, and demonstrate the functional significance of these mutations. The H3K4Me3 peptide is bound in a β-strand conformation in an extensive binding pocket with K4Me3 occupying a deep groove formed by the conserved aromatic residues. A network of intermolecular hydrogen bonds and complementary surface interactions involve six amino-terminal residues of histone H3 (Ala-Arg-Thr-Lys(Me3)-Gln-Thr). We show that the N216, V218 and G221 residues of the ING1 PHD, found mutated in some cancer patients, contribute to the H3K4Me3 recognition. Substitution of these residues diminishes H3K4Me3 binding *in vitro* and impairs the ability of ING1 to induce apoptosis *in vivo*. Together, our results provide novel insights into the molecular mechanism underlying ING1 tumor suppressor activities.

PYRGAKI, C

IMAGING THE NEURAL TUBE CLOSURE IN THE MOUSE EMBRYO C. Pyrgaki (PhD., GS), LA Niswander. Department of Pediatrics University of Colorado, Denver CO

Introduction: Neurulation is the process that gives rise to the central nervous system, and occurs 8.5 days post fertilization for the mouse embryo and around 21 days post fertilization for the human embryos. Disruption of any step of the process of neurulation leads to defects that are called Neural Tube Defects (NTDs) and have a prevalence of one in a thousand live births. The process occurs in three distinct steps and while for the first two steps (elongation of the neural plate and elevation of the neural folds) there is some information available, the last step (neural fold fusion) has been largely unexplored.

Purpose: In the present study we aim to understand how the process of neural fold fusion occurs in mammals. Also we will attempt to determine which step of neural tube development is disrupted in a mouse mutant generated through an ENU mutagenesis screen (FREM2 mutant) and further elucidate the function of this gene in neural tube closure.

Material and Methods: Transgenic mice that express a myristoylated variant of YFP were mated and the females were dissected 8 days after copulation. The embryos were dissected out of the uterus and cultured in media containing 50% freshly prepared rat serum and 50% DMEM, until their neural folds start fusing at the cranial region. Then the embryos were gently pushed out of the yolk sac and positioned properly on a dish placed in

an environmental chamber on the stage of a confocal microscope. The movements of the folds and the behavior of the cells at the fusion points are recorded over a period of 8 to 16 hours. The same experiments were performed on embryos that bear a mutation in the Frem2 gene, and also express the myristoylated Venus protein.

Results: Time-lapse movies during neural tube closure provided us unique insights into neurulation. Multiple fusion initiation points were observed during the fold fusions of the wild type embryos. Cells from the tip of both neural folds protrude towards the opposite fold and extend long, very dynamic cellular protrusions that might contribute to both mechanical and molecular interactions between the two folds. Imaging the Frem2 mutant showed that the first step of neurulation occur normally, but the final step is disrupted.

Conclusions: Our findings suggest that the neural fold fusion might be initiated in multiple points in between the three main fusion points that have so far described. Also the process involves highly dynamic cellular movements that need to be further investigated. Finally the imaging of the Frem2 mutant supported the notion that the exencephalic phenotype is caused by a defect during the last step of the neural tube development

Future directions: We aim to use the powerful system of time lapse imaging of the mouse embryo that we developed to further explore the cellular and molecular mechanisms that drive the neural tube formation. Towards that direction we are planning to use pharmacological agents in the culture media and observe their effect on neural tube formation. Also, we aim to image neural tube formation of a number of exencephalic mutants and use the information of such imaging to identify the part of the process disrupted in each of these mutants to further our understanding of gene function during the critical period of embryogenesis.

ROY, S

REDUCED EXPRESSION OF BOTH p21/CIP1 AND p27/KIP1 PRODUCES A MORE AGGRESSIVE PROSTATE CANCER PHENOTYPE. S Roy, (PhD), Rana Singh, Chapla Agarwal, Sunitha Siriwardana, Robert A. Sclafani and Rajesh Agarwal, Department of Pharmaceutical Sciences, University of Colorado Denver, CO.

Cell cycle deregulation plays a crucial role in cancer progression. In human prostate cancer (PCA), the roles of cyclin dependent kinase inhibitors, p21/Cip1 (p21) and p27/Kip1 (p27) have been studied; however, how they regulate PCA progression is still not clear. Whereas lower p27 protein expression in PCA tissues has often being associated with poor prognosis, the prognostic significance of p21 is still controversial. In order to dissect out the relative importance of both Cip1 and Kip1 proteins in PCA, we generated DU145 cell variants with knocked down levels of p21 (DU-p21), p27 (DU-p27), or both (DU-p21+p27) using retrovirus mediated stable expression of the respective shRNAs and compared their various phenotypic characteristics with empty vector transduced DU145 (DU-EV) cells. Our results showed that only DU-p21+p27 cells have significantly shorter doubling time (28 h) compared to DU-EV (34 h), while DU-p21 and DU-p27 showed similar characteristics as DU-EV with doubling times 34 and 33.5 h, respectively. DU-p21+p27 cells also showed 30% higher ($P<0.001$) clonogenicity compared to DU-EV, but again, no significant effect was observed in DU-p21 and DU-p27 cells. We also examined the *in vivo* tumorigenic potential of these cell lines in athymic nude mice in terms of their xenograft growth for six weeks. Similar to *in vitro* observations, only DU-p21+p27 cells developed much larger tumors (787 mm^3 tumor volume/mouse) than DU-EV cells (276 mm^3 tumor volume/mouse, $P<0.05$); the tumor volumes in case of DU-p21 and DU-p27 were 410 and 355 mm^3, respectively.

Further analyses of tumor samples showed that DU-p21+p27 tumors had a significantly higher proliferation rate, as observed by 54 and 60% increase in proliferating cell nuclear antigen (PCNA) and Ki-67 positive cells compared to DU-EV tumors ($P<0.001$). More importantly, DU-p21+p27 tumors were highly angiogenic as evidenced by higher expression of vascular endothelial growth factor (VEGF), a neo-angiogenesis marker, compared to DU-EV tumors; the expression of PCNA, Ki-67 and VEGF in DU-p21 and DU-27 tumors was comparable to DU-EV controls. Overall, both *in vitro* and *in vivo* results implicate that p21 and p27 have compensatory roles in advanced prostate cancer cells, and that the ablation of both these molecules is necessary for an aggressive prostate carcinoma phenotype.

RUSSELL, TD

LIPID DROPLET SECRETION DEPENDS ON SPECIFIC SURFACE ASSOCIATED PROTEINS. TD Russell (Ph.D., Mol Bio), J Schaack*, JL McManaman**, Departments of Microbiology* and Obstetrics and Gynecology**, University of Colorado Denver Health Sciences Center, Denver, CO.

Accumulation of neutral lipids as cytoplasmic lipid droplets (CLD) is implicated in the pathogenesis of a number of diseases such as cardiovascular disease, diabetes, and cancer and has stimulated interest into the cellular mechanisms controlling formation and secretion of these structures. Since mouse milk is highly enriched in neutral lipids and the lactating mammary gland is one of the most active lipogenic organs in mammals, we use the mouse mammary gland as a model to elucidate these mechanisms. Members of the PAT (perilipin, adipophilin, TIP47) family of lipid droplet associated proteins have been shown to play critical roles in regulating neutral lipid stabilization and contribute to CLD formation and secretion. Previous studies have demonstrated a close functional linkage between the expression of adipophilin in milk secreting cells and the ability of these cells to form and secrete CLD to form milk lipids. In contrast, neither perilipin nor TIP47 localize to CLD in milk secreting cells and their levels do not correlate with milk lipid formation and secretion. These data suggest that adipophilin is the principal PAT protein surrounding CLD in mouse mammary epithelial cells and that it plays key functions in CLD formation and secretion. Sequence analyses and modeling studies have suggested that adipophilin and TIP47 share similar structural properties. In contrast perilipin exhibits only limited sequence homology with these proteins and is predicted to have distinct structural properties. Our goal is to test the hypotheses that formation and secretion of milk lipids dependent upon the presence of ADPH on CLD by investigating how substituting perilipin for adipophilin affects these functions. To accomplish this goal we used utilized a non-invasive intraductal injection technique to transduce milk secreting cells with an adenovirus vector encoding a perilipin-green fluorescent protein (GFP) fusion protein (AdPeri-GFP). Using this technique we found that AdPeri-GFP specifically localized to CLD in milk secreting cells and did not interfere with CLD accumulation during their differentiation. However, we found that CLD secretion by these cells was profoundly impaired during lactation. These results suggest that although other PAT proteins can substitute for adipophilin in CLD formation, the secretion of CLD appears to depend on specific structural properties of ADPH. We are currently adapting novel methods such as flow cytometry and laser capture microscopy to investigate the specific effects of perilipin on CLD properties and lipid synthesis gene expression in milk secreting cells.

SCHAMBERS, L

UTILIZATION OF A RANDOM PEPTIDE LIBRARY TO SCREEN RECOMBINANT ANTIBODIES GENERATED FROM MULTIPLE SCLEROS IS CEREBROSPINAL FLUID. L. Schambers, (M.D., SOM), X. Yu, Department of Neurology, University of Colorado, Denver, CO

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease affecting the central nervous system (CNS). Intrathecal synthesis and presence of oligoclonal immunoglobulin (Ig) G in the brain and cerebrospinal fluid (CSF) is often used as a diagnostic marker for this autoimmune disorder. However, the specificity of oligoclonal IgG in MS is unknown. Previous experimentation demonstrates that monoclonal recombinant antibodies (rABs) produced from single plasma cells from MS CSF can be probed by screening a phage-displayed random peptide library to potentially identify continuous epitopes or mimotopes of MS antigens (Yu et al., 2006).

The purpose of this study was to probe two distinct rABS (#1 and #2) generated from the CSF of one MS patient (MS03-1) using a phage-displayed random peptide library with 12-mer peptides. This was done through three rounds of panning, after which affinity-selected peptides were identified, and ELISA was used to confirm the binding specificity. Affinity selected phage peptides from both rABs were sequenced and aligned to the Swiss-Prot database. Both rABs #1 and #2 demonstrated high affinity for a number of peptides illustrating the effectiveness of random peptide library screening in determining the binding specificity of rABs. Probing rABs from various MS patients and comparing the specifically selected peptide sequences may help to identify a common binding pattern or a common antigen among MS patients.

SHEEDER, J

FIFTEEN YEARS LATER: ADOLESCENT MOTHERS WEIGH MORE BUT THEIR BABIES DO NOT (1990 TO 2005). Jeanelle Sheeder MSPH (PhD – Clinical Science), Catherine Stevens-Simon MD. Department of Pediatrics University of Colorado School of Medicine; Denver, Colorado.

PURPOSE: American adolescents are bigger and more likely to be overweight or obese than they were two decades ago. Adult mothers and their infants have also gotten bigger. The purpose of this study was to determine if the size of pregnant adolescents and the size and maturity of their infants has changed.

METHODS: A racially/ethnically diverse group of 1,187, 13-through-18 year old, primigravida participants in an adolescent-oriented maternity program was enrolled consecutively between 1990 and 2005 and grouped by year of conception. Maternal outcomes were: self-reported preconception weight, measured height, body mass index (BMI; weight/height^2), and the proportion of under, average, and overweight/obese adolescents. Infant outcomes were: birth weight, gestational age, and the proportion of small, average, and large for gestational age and preterm births. Covariates included: age, race/ethnicity, smoking, pregnancy induced hypertension (PIH), abnormal glucose screen, Caesarian delivery, labor induction, and infant sex. The analysis used ANOVAs/MANOVAs.

RESULTS: The ANOVAs revealed a significant increase in maternal weight ($p=0.006$), BMI ($p=0.002$), overweight/obese status ($p<0.0001$), age ($p<0.0001$), Hispanic ethnicity ($p<0.0001$), and induced labors ($p=0.004$) over the study period. There was also a significant decrease in maternal height ($p<0.0001$), PIH ($p=0.01$), and Caucasian adolescents ($p<0.0001$). None of the infant outcome measures or other covariates changed significantly over the 15 years. Following adjustment for significant covariates (age and race/ethnicity),

the increase in maternal weight and decrease in height remained statistically significant (p=0.04 and 0.01, respectively). None of the other maternal or infant outcome measures changed significantly.

CONCLUSIONS: The weight of adolescent mothers has increased over the past 15 years but neither the weight of their infants nor the proportion of small-for-gestational age and preterm births has changed. The findings are reminiscent of the results of studies demonstrating that adolescents give birth to smaller infants than same-sized adults and transfer less of the weight they gain during gestation to their fetuses.

SHIELDS, KM

DEFICIENCY OF MAP KINASE PHOSPHATASE-1, A CRITICAL INFLAMMATORY MODULATOR, INDUCES DEVELOPMENT OF PULMONARY HYPERTENSION IN MICE. KM Shields (MD, SOM), J. Harral, M. Oka, N. Homma, N. Burns, J. West, M. Das. Department of Pediatrics, Critical Care, University of Colorado Denver, CO

MAP Kinase Phosphatase-1 (MKP-1), a deactivator of MAP kinases, is an important inflammatory regulator. However, the role of MKP-1 in pulmonary hypertension is unknown. We hypothesized that MKP-1 deficiency will contribute to development of pulmonary hypertension through deregulation of the MAPK pathway.

MKP-1 null (MKP-$1^{-/-}$), heterozygous (MKP-$1^{+/-}$), and wild type (MKP-$1^{+/+}$) mice (4-5 weeks old) (Lexicon Genetics Incorporated) were exposed to sea level (SL), Denver altitude (DA; 5280 feet), or severe altitude (HYP; 17,000 feet) for 6 weeks and then assessed for weight gain, cardiac hypertrophy measured by right ventricle to left ventricle + septum wet weight ratio(RV/ LV+S), and right ventricular systolic pressure (RVSP) via cardiac catheterization. Lung sections were stained for alpha smooth muscle actin (SMA), phosphoERK1/2, phosphoJNK, phosphop38, and COX-2.

MKP-$1^{+/-}$ had the least weight gain after 6 weeks SL exposure. HYP inhibited weight gain in MKP-$1^{+/-}$ and MKP-$1^{-/-}$. RV/LV+S was increased by HYP in all groups RVSP was highest in MKP-$1^{+/-}$ mice after SL exposure. RVSP was higher in DA and HYP than SL in MKP-$1^{-/-}$ mice. SMA staining was increased in the HYP lungs of MKP-$1^{+/-}$ and MKP-$1^{-/-}$ mice. Vessel to alveoli ratio was decreased in HYP lungs of MKP-$1^{+/-}$ and MKP-$1^{-/-}$ mice. Vessel (<50μm) wall thickness to diameter ratio increased in MKP-$1^{+/-}$ and MKP-$1^{-/-}$ lungs. Immunoreactivity against phosphoERK1/2 and phoshpoJNK was similar in all groups. Phosphop38 staining intensity was highest in the MKP-$1^{-/-}$ lungs with positive reaction in vessels, respiratory epithelium, and interstitial cells. COX-2, which is induced during inflammation, also had strongest staining in the MKP-$1^{-/-}$ lungs.

Lack of MKP-1 causes sustained p38 activation in the lung, which may be a major contributor in the development of pulmonary hypertension.

SPARKS, T

DEPRESSED MOTHER'S PERCEPTIONS OF THEIR MASTERY MOTIVATION. T. Sparks (M.D., MS), S. Hunter, T. Backman, and R. Ross, Psychiatry Scholars Program, Department of Psychiatry & Behavioral Sciences, University of Colorado, Denver, CO.

Maternal depression has been shown to influence child behavior and development, including aspects of mastery motivation. Mastery motivation is a "psychological force that stimulates an individual to attempt independently, in a focused and persistent manner, to solve a problem or master a skill or task that is moderately challenging for him or her[1]." This study will examine mastery motivation in infants of mothers with and without

depressive symptoms. Additionally, as this study's measure of mastery motivation is a parent-report measure and since maternal depression may alter perceptions of child behavior, this study will compare mothers' ratings of their infant's mastery motivation with the Behavior Rating Scale (BRS) of the Bayley Scales of Infant Development.
Methods. Infants' mastery motivation will be assessed by having the mother complete the Dimensions of Mastery Questionnaire (DMQ) at six, twelve, and eighteen months of age. Mother's current level of parental distress will be assessed at each of these visits using the Parenting Stress Index Short Form (PSI-SF). Additionally the BRS will be completed at each of the three visits.
Results. Correlation analyses will be used to evaluate the relationship between mothers' level of parental distress (PSI-SF), mothers' assessment of infants' mastery motivation (DMQ), and a rater's assessment of infants' performance (BRS).
Conclusion. We hypothesize that higher parental distress subscale scores on the PSI will be negatively correlated with infant DMQ scores. We further hypothesize that observer ratings will be higher than maternal ratings of infant behavior.
[1.] Morgan, G., Harmon, R., and C. Maslin-Cole. (1990). Mastery Motivation: Definition and Measurement. *Early Education and Development*, 1:318.

STEPHENS, SH & FRANKS, A

ASSOCIATION OF THE 5' UPSTREAM REGULATORY REGION OF THE *α7 NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT GENE* (*CHRNA7*) WITH SCHIZOPHRENIA.
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The α7 neuronal nicotinic acetylcholine receptor subunit gene (*CHRNA7*) is localized in a chromosomal region linked to schizophrenia in multiple independent studies. *CHRNA7* was selected as the best candidate gene for a well documented endophenotype of schizophrenia, the P50 sensory processing deficit, by both genetic linkage to the locus and human and animal studies. Mutation screening of the *CHRNA7* coding region and intron/exon splice junctions revealed multiple synonymous variants and rare non-synonmous variants that were not associated with schizophrenia or the P50 deficit. A large number of functional mutations in the proximal promoter were more abundant in schizophrenics compared to controls. The presence of promoter polymorphisms was also associated with the P50 sensory processing deficit in control subjects. The current study investigated additional SNPs in the 5'-upstream regulatory region of *CHRNA7* for association with schizophrenia and smoking in schizophrenia. Family-based and case-control association studies were performed on samples from 123 families as well as 348 individual schizophrenic patients and 144 controls. The rs3087454 SNP, located in the upstream regulatory region of *CHRNA7*, was significantly associated with schizophrenia in the case-control sample, and association was supported in family members. This SNP was also associated with smoking in the disorder.

SUÁREZ, AL

ENDOTHELIAL CELLS SUPPORT PERSISTENT GAMMAHERPESVIRUS INFECTION IN VITRO. AL Suárez, (MD/PhD, SOM, GS), LF van Dyk, Departments of Molecular Biology, Microbiology, and Immunology, University of Colorado Health Sciences Center, Aurora, CO.

A variety of human diseases are associated with gammaherpesviruses, including neoplasms of lymphocytes (e.g. Burkitt's lymphoma) and endothelial cells (e.g. Kaposi's sarcoma). Many of these diseases predominately occur in immunocompromised individuals (e.g. AIDS patients). Gammaherpesvirus infections usually result in either a productive lytic infection, characterized by expression of all viral genes and rapid cell death, or latent infection, characterized by limited viral gene expression and no cell death. Here we report characterization of endothelial cell infection with murine gammaherpesvirus 68 (γHV68), a virus phylogenetically related and biologically similar to the human gammaherpesviruses. While endothelial cells supported γHV68 replication in vitro, these cells were unique in that a significant proportion of the cells escaped destruction, proliferated, and remained viable in culture for an extended time. In contrast to uninfected endothelial cells, these cells were non-adherent and altered in size, complexity, and cell-surface protein expression. These cells were uniformly infected based on detection of viral protein expression, synthesis of GFP from the viral genome, and abundant viral gene transcripts. Additionally, endothelial cells continued to produce new infectious virions as late as 30 days post-infection. The outcome of this long-term infection was promoted by the γHV68 viral cyclin, because in the absence of this viral gene, endothelial cell viability was reduced following infection. Prolonged viability of infected endothelial cells, combined with continued infectious virus production for weeks after infection indicate that endothelial cells can support persistent gammaherpesvirus infection in vitro. These data provide an additional mechanism, beyond latency, by which gammaherpesviruses can achieve long-term propagation. Currently we are testing the in vivo role of endothelial cells in γHV68 infection of both wildtype and immunodeficient mice. Preliminary data indicate that endothelial cells, unlike other cell types studied to date, maintain expression of major histocompatibility complex class I during γHV68 infection. These findings suggest that infected endothelial cells, which would otherwise be cleared by an intact host immune system, could serve as a source of persistent γHV68 infection in the context of immunodeficiency.

TRAN, H

GENETIC ALTERATIONS IN CCNE1 3' UTR LEAD TO ALTERED GENE EXPRESSION. Tran, H., (M.D., SOM) Chen, R., Bashir, Q., Amato, C., Bemis, L., Robinson, W. Department of Oncology, University of Colorado Denver, School of Medicine.

STUDY OBJECTIVES

1) Determine if there are differential expression of CCNE1 in 4 MCL cell lines (Granta, Jeko, JVM, Z138)

2 Determine if there are mutations within the 3' UTR of CCNE1 in 4 MCL cell lines and if they alter miRNA binding sites

3) Determine if mutations alter mRNA and/or protein expression

METHODS

1) Western blot was used to analyze CCNE1 protein expression. Antibody to CCNE1 was purchased from Abcam. Antibody to B-Actin was purchased from Cell Signalling.

2) A Bioinformatics approach was used to identify predicted miRNA binding sites in the CCNE1. The predicted miRNAs are miR-15, miR-16, miR-34, and miR-497. DNA and RNA were extracted from4 MCL cell lines. Primers for PCR were designed to span CCNE1 3' UTR. DNA was PCR amplified, gel extracted, and sequenced.
3) Primers for qRT-PCR was designed to the coding region exons 2-4 to avoid DNA contamination. qRT-PCR was performed to study CCNE1 mRNA expression.

FINDINGS

1) There is differential CCNE1 protein expression in MCL cell lines. Z138 and Jeko have the highest CCNE1 expression.
2) Three different mutations were found in CCNE1 3' UTR. The first mutation is a homozygous C→T at 19bp region just prior to the start of the 3' UTR (Granta homozygous, JVM heterozygous). The second mutation is a heterozygous C→T mutation at 285 bp region in Granta. The third mutation is a heterozygous C→A mutation in the 428 bp region in Z138.
3) There is differential expression of total CCNE1 mRNA in 4 MCL cell lines.

CONCLUSIONS

There are mutations in CCNE1 3' UTR. These mutations are not in the miRNA binding sites as predicted by bioinformatics. However, they may still have indirect effects on miRNA binding due to alterations in RNA folding patterns. What can be implied from the qRT-PCR and western blot data is that the C→ T substitution at the 19 bp region may downregulate mRNA expression and the C→ A substitution at 428 bp region may increase translational inhibition. Further investigations such as reporter constructs are needed to fully characterize the exact biological effects of these mutations. Also, investigations into CCDN2, D3, and E2 will be done as well to look for genetic alterations.

VITELLO, AM

ANTIBODY MICROARRAY ANALYSIS OF FAILING HUMAN HEART VENTRICLES REVEALS NOVEL SIGNALING PROTEIN ABNORMALITIES. AM Vitello (MD, SOM), Y Du, P Buttrick, LA Walker, Department of Cardiology, University of Colorado, Denver, CO.

It is thought that several biochemical signaling pathways contribute to heart failure disease progression including those involved in inflammation, apoptosis, and proliferation. The purpose of this study was to elucidate the global proteomic changes seen in heart failure in order to approach the pathophysiology of this disease in both right (RV) and left (LV) ventricles at a molecular level. Human tissue samples were obtained from both control and failing right and left ventricles. Samples were homogenized, protein concentration was measured, and an equal amount of protein sample was labeled with either cyanine 3 or cyanine 5 fluorophores. Labeled homogenates were desalted to removed excess unbound dye and protein concentration and labeling efficacy was determined. Samples were mixed to provide the following comparisons: control RV vs. control LV, control RV vs. failing RV, control LV vs. failing LV, failing RV vs. failing LV. Protein mixtures were incubated with an antibody microarray (Clontech). Microarrays were imaged using a Perkin Elmer microarray scanner and relative cyanine 5 to cyanine 3 fluorescence was determined. Changes in the cyanine 5 to cyanine 3 ratios were analyzed among 505 different proteins. Significant protein differences that were observed were validated using western blot analysis. Our antibody microarray findings indicate that there were no significant protein differences between control RV compared to control LV. However, IL-1b and DMPK were upregulated in failing RV compared with control RV; Caspase-9/ ICE – LAP6/ Apaf-3 and FADD/ Mort-1 were downregulated in failing LV

compared to control LV; NF-kB, doublecortin, PARP, and nexilin were upregulated in failing LV compared to control LV; p96 and flotillin2/ ESA were upregulated in failing RV compared to failing LV; and CSK and cytochrome c/ Apaf were upregulated in failing LV compared to failing RV. Preliminary western blot data confirm several of the microarray findings. The proteins that were found to be upregulated are involved in growth, proliferation, and apoptotic signaling pathways. This could potentially explain certain gross pathologic changes that take place in the heart in the setting of heart failure, including hypertrophy and fibrosis. Future studies may include probing for other molecules in the various signaling pathways. By elucidating these mechanisms it may be possible to design chamber specific therapies that might ameliorate the pathologic changes observed in heart failure.

VOLKERT, E

IMPACT OF MATERNAL DISTRESS ON PEDIATRIC PRIMARY CARE.

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Postpartum mood disorders have been shown to negatively affect child development, parenting behaviors, and child health outcomes. Early identification of postpartum mood disorders by physicians offers an opportunity for intervention and treatment. Yet prior studies show that the majority of pediatricians do not screen for postpartum mood disorders and that postpartum depression is commonly underdiagnosed.

The goal of this study was to provide a descriptive overview of how maternal distress relates to pediatric primary care and health outcomes in an inner-city, pediatric clinic.

Data was collected from 112 mothers and children visiting the clinic for the child's 12, 18, and 24 month well-child visits. Mothers were screened for depressive symptoms using the Edinburgh Postnatal Depression Scale (EPDS). Data on child development, parenting skills, and socioeconomic health were collected using the Ages and Stages Questionnaire: Social-Emotional, Life Skills Progression Survey, Early Childhood Screening Assessment, and Parents Evaluation of Developmental Status.

Children of mothers with greater depressive symptoms were less likely to attend well-child visits and scored more poorly in the Ages and Stages Questionnaire for social and emotional development. Additionally, the gap between children of mothers with low depressive symptoms versus high depressive symptoms increased progressively with age, suggesting a cumulative effect on social and emotional development.

These results are consistent with findings from previous studies and offer additional evidence for a cumulative negative effect of maternal distress on child outcomes. Methods incorporated in this study, including the EPDS, suggest a means of screening for maternal distress in the context of routine pediatric care.

Maternal distress is common, underdiagnosed, undertreated, and has serious health consequences for children. A better understanding of how maternal distress manifests in pediatric care will help to improve detection and treatment of maternal depression and improve family outcomes.

WILSON, N

DEPTH-DEPENDENT PHOTOPOLYMERIZATION REACTION KINETICS IN DENTAL COMPOSITES

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Several clinically important properties of dental composite materials are known to be highly dependent on degree of conversion. Many studies have examined the depth of cure of photocured composites with some reporting results based on physical probing and others measuring either top vs. bottom or even cross-sectional depth-dependent hardness or conversion. Objectives: Since no information is available on dynamic reaction kinetics and corresponding thermal profiles as a function of depth within a photopolymerizing composite, real-time measurement of these factors in composite materials were conducted. Because the rate and potentially the magnitude of stress development during polymerization depend on the rate of polymerization, a better understanding of local reaction kinetics could provide useful insights regarding dentin bonding as well as offering a novel, more rigorous method for depth of cure evaluations in composite materials. Methods: A resin composition of Bis-GMA and triethylene glycol dimethacrylate containing camphorquinone and ethyl 4-dimethylaminobenzoate as the visible light photoinitiator system was combined with 70 wt% silanized barium glass filler to provide a representative dental composite. The composite paste was packed into a transparent thin-walled Teflon cylinder (3 mm inner diameter x 12 mm length) which was positioned vertically in the near-infrared (NIR) spectrometer beam, which was used to assess real-time conversion within the specimen during photopolymerization. An aperture was used to restrict the NIR signal access to a 1 mm horizontal slice at a predetermined depth in the composite specimen. An embedded thermocouple was positioned just below the NIR sampling level to provide dynamic localized temperature sensing to accompany the NIR conversion data. A 90 s exposure to a visible wavelength dental curing light providing either 50 or 500 mW/cm^2 incident irradiance to the open upper surface of the composite was used to trigger the polymerization. Results: The conversion reached at the end of the exposure interval varied linearly with the sampling depth between 1 and 9 mm: r^2 values of 0.97 and 0.98 for the 50 and 500 mW/cm^2 conditions, respectively. At all depths, the lower irradiance exposure produced significantly lower conversion ($p < 0.05$) which decreased more rapidly with respect to depth compared with the higher irradiance. The dynamic conversion data during polymerization demonstrate that internal temperature and reaction rate both decrease with increasing depth within the specimen, but with the thermal front advancing ahead of the reaction process. At the 9 mm sampling depth, delays of > 10 s and > 40 s were observed between the onset on sample exposure and local polymerization at the high and low light intensities, respectively. Conclusions: This novel analytical approach to reaction monitoring as a function of composite depth comprehensively demonstrates the significant effects of attenuated light transmission on photopolymerization in these materials.

YONKERS, MA

THYROID HORMONE RAPIDLY AFFECTS VOLTAGE-GATED SODIUM CURRENT IN ZEBRAFISH EMBRYONIC NEURONS VIA AN INTEGRIN RECEPTOR. MA Yonkers (MSTP), AB Ribera, Department of Physiology, University of Colorado Denver - AMC, Aurora, CO.

Vertebrate neurodevelopment requires thyroid hormones, yet the underlying mechanisms are not well defined. The clinical deficits resulting from human thyroid hormone deficiency during development suggest that thyroid hormones regulate key neuronal properties. One key neuronal property, voltage-gated sodium current (I_{Na}),

underlies rapid signaling in neurons and alterations in I_{Na} affect neurodevelopment. If thyroid hormones regulate I_{Na} during embryonic stages, then thyroid hormone concentrations would regulate both rapid signaling and neurodevelopment. We tested how thyroid hormones affected the I_{Na} of a primary sensory neuron, Rohon-Beard (RB) cells, in the zebrafish (*Danio rerio*) embryo at 50-55 hours post fertilization (hpf). Using whole-cell patch clamp methods, we found that the conventionally active thyroid hormone triiodothyronine (T3) had no effect on RB I_{Na}, but its precursor thyroxine (T4) significantly increased peak I_{Na} within five minutes of application by 39% over control ($p < 0.05$). Co-administration of a competitive thyroid hormone inhibitor (tetrac) with T4 prevented the rapid increase in peak I_{Na} ($p < 0.05$), indicating that the effect required a thyroid hormone receptor. In contrast, acute T4 did not affect voltage-gated potassium current. The specificity of T4 and the rapid time course of action suggested a nongenomic mechanism for thyroid hormone modulation of I_{Na}. Recently, integrin receptors were implicated in nongenomic thyroid hormone signaling *in vitro*. We tested whether RB neurons expressed the integrin receptor dimer αvβ3 during 50-55 hpf. Immunofluorescent staining showed expression of αvβ3 protein on RB cells during 50-55 hpf in the dorsal spinal cord of wildtype embryos. However, zebrafish embryos did not stain for αvβ3 earlier in development at 24 hpf. We then injected the RGD-type integrin blocker echistatin or the αvβ3 function-blocking antibody LM609 into zebrafish embryos to assay for the role of integrins in T4 signaling. Both echistatin and LM609 occluded T4's acute regulation of I_{Na} ($p < 0.05$), further supporting a nongenomic T4-integrin mechanism. As a control, injection of vehicle only (H_2O) recovered the acute T4 increase in I_{Na}. Additionally, T4 had no acute effect on RB I_{Na} when tested at 24 hpf, when αvβ3 is not expressed on RB cells, consistent with αvβ3 acting as a T4 receptor on RB neurons. Overall, our results show that T4 can act separately from T3 in the embryonic nervous system, highlighting the importance of prenatal T4 concentrations, and suggest a receptor target for treatment of thyroid hormone disorders that may involve nongenomic mechanisms.

YOUNG, CD

ACTIVATED AKT1 ACCELERATES MAMMARY TUMORIGENESIS IN MMTV-C-ERBB2 TRANSGENIC MICE WITHOUT OVEREXPRESSION/ACTIVATION OF ERBB3. CD Young (PhD, GS), E Nolte, A Lewis, N Serkova, SM Anderson. Department of Pathology, University of Colorado Denver.

ErbB2, a member of the epidermal growth factor receptor family (EGFR), is overexpressed in 20-30% of human breast cancer cases and forms oncogenic signaling complexes when dimerized to ErbB3 or other EGFR family members. We have crossed the MMTV-myr-Akt1 transgenic mice (which express constitutively active Akt1 in the mammary gland) with MMTV-c-ErbB2 transgenic mice to evaluate the role of Akt1 activation in ErbB2-induced mammary carcinoma. Bitransgenic MMTV-c-ErbB2, MMTV-myr-Akt1 mice develop mammary tumors in 114 days as compared to 231 days in the MMTV-c-ErbB2 mice. Histology and activated caspase 3 immunohistochemistry demonstrate that the bitransgenic tumors are more aggressive, more necrotic, less organized, and less apoptotic than the tumors from MMTV-c-ErbB2 mice.

The two tumor types demonstrate dramatically different expression and activation of EGFR family members as well as different metabolic profiles. c-ErbB2 tumors demonstrate overexpression of EGFR, ErbB2, ErbB3 and ErbB4 as well as activating phosphorylation of ErbB2 and ErbB3, underscoring the importance of the entire EGFR family in ErbB2-induced tumorigenesis. Tumors from bitransgenic mice demonstrate

overexpression of the myr-Akt1 and ErbB2 transgenes, however there is dramatically less overexpression and phosphorylation of ErbB3, decreased levels of EGFR and ErbB4 proteins, decreased ErbB2 phosphorylation and decreased downstream tyrosine phosphorylation as compared to the c-ErbB2 tumors. Thus, activation of Akt1 alters the requirement for EGFR family signaling in c-ErbB2-induced tumorigenesis. The bitransgenic tumors contain more lactate as well as the glucose transporter, GLUT1, indicating increased glycolysis and glucose transport. However, GLUT1 is being induced by hypoxia rather than by activation of Akt.