

UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS

26TH ANNUAL STUDENT RESEARCH FORUM

AND

STUDENT RESEARCH AWARDS CONVOCATION

COLLEGE OF NURSING

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DECEMBER 13, 2011
ANSCHUTZ MEDICAL CAMPUS
EDUCATION 2 NORTH/SOUTH

26TH ANNUAL
UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS
STUDENT RESEARCH FORUM

Tuesday, December 13, 2011

Poster Sessions

1:00-2:15 pm

2:15-3:30 pm

Awards Convocation

4:30 pm

ED2 South Room1102

ANSCHUTZ MEDICAL CAMPUS
EDUCATION 2 NORTH/SOUTH

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ABSTRACTS

ANTONIOLI, A

Evaluation of siRNA Knockdown of MASP-1 in the Alternative Pathway of Complement Analysis of factors that regulate different pathways of the complement system is important for understanding its role in overall host immune defense and dysregulation in diseases such as rheumatoid arthritis (RA). The complement system consists of a classical pathway (CP), alternative pathway (AP) and lectin pathway (LP). The AP does not require any specific molecular recognition by its early molecule(s) for its initiation but is rather activated by spontaneous hydrolysis of C3 to C3 (H2O). An important component of the AP is complement factor D (Df), which cleaves C3b-bound factor B into Ba and Bb to generate C3bBb, the AP C3 convertase. Studies show that activation of the AP is highly dependent on formation of proteolytically active Df by cleavage of a five amino acid peptide from its inactive zymogen form (pro-Df). Recently it has been shown that binding of MASP-1/3 proteins facilitates cleavage of pro-Df to mature active Df. The latter is required for the activation of the AP. Mice genetically deficient in MASP-1/3 proteins are unable to cleave Pro-Df and fail to activate the AP. The relevance of these results is supported by an antibody-induced arthritis (CAIA) model that is mainly dependent on the activation of the AP. Deficiency of MASP-1/3 in the CAIA model decreases deposition of C3 and generation of C5a and shows significant impairment of the AP. Studies by Banda et al. (2011) also suggest that pathogenic activation of the AP through pro-Df cleavage may occur locally through release of MASP-1 from cells intertwined with adipose cells specifically fibroblast-like synoviocytes (FLS) and macrophages which are present in the synovium. Here we address whether sequence-specific silencing of MASP-1 with siRNA can be useful for examining the local mechanism and importance of MASP-1-mediated cleavage of pro-Df into mature Df. Given that MASP regulation of pro-Df has been studied only with MASP-1/3 knockout mice, sequence specific local tissue knockdown of MASP-1 and possibly MASP-3 separately would help clarify how these two factors are involved in the activation and regulation of the AP. In vitro experiments with siRNA-MASP-1 have indicated that there is some downregulation of MASP-1 activity. These results suggest that the conversion of pro-Df to mature Df may be altered and AP activation may be impaired.

ARNETT, D

NEOADJUVANT CHEMOTHERAPY PRIOR TO CYSTECTOMY IN PT2 PATIENTS – THINK TWICE?

DM Arnett (MD, SOM), S Wilson MD, Department of Urology, University of Colorado
INTRODUCTION AND OBJECTIVES: The role of neoadjuvant chemotherapy (NAC) with cystectomy is controversial. Although many studies have shown no advantage, a large, prospective randomized trial has shown an overall survival benefit to the administration of NAC with cystectomy. The study population for these studies included patients with a broad range of cancer stages (T0-T4a). Evaluation of results from a homogeneous clinical population may inform more individualized treatment decisions. We sought to evaluate the outcomes of patients treated neoadjuvantly with stage T2 bladder cancer as compared to radical cystectomy alone in a large-volume university setting. **METHODS:** Medical records were reviewed for 186 patients who underwent cystectomy for cancer by a single surgeon during a 68 month period at our institution. Patients with pathological staging of T2 pathology on cystectomy or radical transurethral resection of bladder tumors (if final pathology revealed T0 disease) were included for analysis (n=95). No patient had cancerous nodal involvement. Kaplan-Meier survival curves were generated for both cohorts and tested for a difference with the logrank test. As a shift to NAC was not experienced until 2009, only 21 of these patients received NAC prior to surgery and 74 patients underwent cystectomy alone. Patient follow-up continued between 0 and 57 months post-surgery (M = 18 months). There was no obvious selection bias for patients undergoing NAC. **RESULTS:** Surprisingly, T2 Patients who underwent combination therapy had a significantly worse survival rate than patients who underwent radical cystectomy alone (logrank: p

ARNETT, D

PARTIAL CYSTECTOMY FOLLOWED BY RADICAL CYSTECTOMY - HOW DO THESE PATIENTS

DO? DM Arnett (MD, SOM), C O'Donnell PhD, S Wilson MD, University of Colorado
INTRODUCTION AND OBJECTIVES: Research on the effectiveness of partial versus radical cystectomy reports no difference in survival rates in carefully selected patients. In our practice we frequently perform radical cystectomy on patients who have previously undergone partial cystectomy (PC). As approximately 25% of patients who undergo PC ultimately require radical cystectomy, and as there is the possibility of spillage of tumor with PC, we hypothesized that patients who undergo PC for transitional cell carcinoma (TCC) followed by radical cystectomy have worse outcomes than patients treated with immediate radical cystectomy alone. **METHODS:** 170 patients with TCC who were treated successively by a single surgeon at the University of Colorado Hospital between December 2004 and July 2010 were identified. Ten patients had undergone prior PC. Although the numbers were not large in the prior PC group, we hypothesized that the difference in outcome might be large. Follow-up was obtained on all patients and documented with a mean follow-up of 17 months (range 1 to 122). **RESULTS:** Results were tabulated using a logrank test on the Kaplan-Meier survival curves. There was no significant difference in survival between patients who had undergone PC and those who had not prior to definitive therapy (p=.57). Overall 2-year survival in patients with previous PC was 80%, compared to 75% for patients who had not undergone prior PC. Interestingly, the cause of death was related to bladder cancer in 10% of patients with a prior PC (n=1), but 17% of patients who had not had prior PC before radical cystectomy (n=27). These data suggest that with a larger sample size, a difference may ultimately be seen in patients who have had prior PC. A selection bias is an obvious explanation for this possible advantage. **CONCLUSIONS:** Partial cystectomy prior to radical cystectomy does not portend a statistically significant poorer overall survival. There may be a trend toward poorer disease-specific survival, but this has not yet reached statistical significance. Although patients who qualify for PC are rare, at this time they may be counseled that this choice most likely will not reduce their overall survival if ultimately radical cystectomy is required.

BATES, C

GAS6-INFLUENCED EFFECTS ON MER GLYCOSYLATION AND NUCLEAR EXPRESSION. CA

BATES (MD, SOM), J MIGDALL, J SCHLEGEL, RMA LINGER, D DERYCKERE, DK GRAHAM,
DEPARTMENT OF PEDIATRICS, UNIVERSITY OF COLORADO, DENVER, CO. MER RECEPTOR
TYROSINE KINASE IS ECTOPICALLY EXPRESSED IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL),
THE MOST COMMON PEDIATRIC MALIGNANCY. ECTOPIC MER EXPRESSION IS ASSOCIATED
WITH LEUKEMOGENESIS AND INCREASED CHEMORESISTANCE, AS WELL AS MORE
AGGRESSIVE AND RELAPSED FORMS OF ALL. THESE EFFECTS ARE LARGELY BELIEVED TO
OCCUR THROUGH INCREASED PRO-SURVIVAL SIGNALING ACTIVITY, WHICH HAS BEEN
DEMONSTRATED IN VITRO DOWNSTREAM OF MER STIMULATION BY ITS LIGAND, GAS6.
INTERESTINGLY, MER-EXPRESSING LYMPHOCYTES HAVE BEEN SHOWN TO PROMOTE
OSTEOBLAST SECRETION OF GAS6, ULTIMATELY LEADING TO A BONE MARROW
MICROENVIRONMENT THAT IS RELATIVELY ENRICHED IN THIS LIGAND. THE CURRENT STUDY
ADDRESSES LONGER-TERM CONSEQUENCES OF THIS GAS6-REplete ENVIRONMENT ON MER
IN SEVERAL HUMAN LEUKEMIC CELL LINES. EFFECTS OF PROLONGED STIMULATION OF MER
WERE ANALYZED USING FLOW CYTOMETRY, CELLULAR FRACTIONATION, WESTERN BLOT,
AND IMMUNOFLUORESCENCE MICROSCOPY. LONG-TERM EXPOSURE TO GAS6 FAVORED THE
PRODUCTION OF A PARTIALLY N-GLYCOSYLATED FORM OF MER THAT AROSE FROM A NEWLY
SYNTHESIZED PROTEIN. THIS CHANGE WAS ASSOCIATED WITH DECREASED SURFACE
LEVELS OF MER AS WELL AS ALTERED LOCALIZATION OF MER IN THE NUCLEAR-SOLUBLE AND
CHROMATIN-BOUND FRACTIONS IN A GLYCOFORM-SPECIFIC FASHION. THE PRESENCE OF
MER IN THE NUCLEUS IS A NOVEL FINDING. THE ALTERED LOCALIZATION OF THE DIFFERENT
MER GLYCOFORMS SUGGESTS THAT GLYCOSYLATION MAY AFFECT MER FUNCTION WITHIN
EACH NUCLEAR COMPARTMENT. BEYOND ENHANCING OUR UNDERSTANDING OF MER, THESE
FINDINGS ALSO BROADEN THE SCOPE OF POTENTIAL TARGETS AVAILABLE FOR THE
DEVELOPMENT OF MER-SPECIFIC INHIBITORS.

GAIN AND LOSS OF LIVER X RECEPTOR FUNCTION STUDIES SUGGEST A POTENTIAL BUT COMPLEX ROLE IN BONE. AR Berry (M.D., GS), LC Shum, RM Ackerson, KB King, *Department of Orthopaedics and Bioengineering, University of Colorado School of Medicine, Aurora, CO.*

Purpose: The liver X receptor (LXR) is a nuclear receptor that functions in oxysterol metabolism in the liver and other tissues. A role for oxysterols in osteoblast (OB) differentiation has been proposed. We investigated if activation of LXR could benefit bone health. We used *in vivo* loss-of-function (LOF) and gain-of-function (GOF) experiments to test this hypothesis. Methods: (1) To investigate LOF, LXR KO mice were generated. Mice deficient in the α (α KO), β (β KO), or both isoforms (α/β KO) were compared to WT. (2) To investigate GOF, mice were fed chow containing DMHCA (LXR activator) for 2 weeks. Control mice were fed normal chow. Gene expression levels for LXR α , LXR β , collagen type 1 (Col1A1), osteocalcin (OCN), alkaline phosphatase (ALP), and bone sialoprotein (BSP) were quantified using qRT-PCR. Fold-changes in mRNA expression were determined with the $2^{-\Delta\Delta C_t}$ data analysis method (Fig. 1). (3) Human pre-OB cells were cultured with and without DMHCA for 3, 7 and 14 days to measure OB differentiation and function. OB mRNA was extracted and quantified for two LXR downstream targets: ABCA1 and ABCG1, as well as OCN, an OB differentiation marker. Statistically significant differences between groups were determined. Results: (1) There was a large decrease in β in both β KO and α/β KO (738 and 224-fold, $P < 0.05$), but no difference in LXR α . Bone-related targets were increased in α KO bone but were decreased or without change in β KO and α/β KO bone. (2) DMHCA treatment gave a small non-significant decrease in bone targets. (3) DMHCA treatment increased ABCA1 and ABCG1 at 7d and 14d, and OCN at 7d (Fig. 2). Conclusions: LXR plays a complex role in bone. Systemic inactivation of α stimulates bone genes. Bone has low levels of the α form when compared to β . Negative effects on bone from systemic LXR α activation may be downstream of strong LXR activation elsewhere (liver for example). Paradoxically, *in vitro* DMHCA treated OBs suggest that local activation of LXR β may be beneficial to bone health. LXR activation may have differential effects on bone health when activated systemically (Fig. 1) versus locally (Fig. 2). Thus, activation of LXR β at sites of local pathology may be a tool for improving bone care.

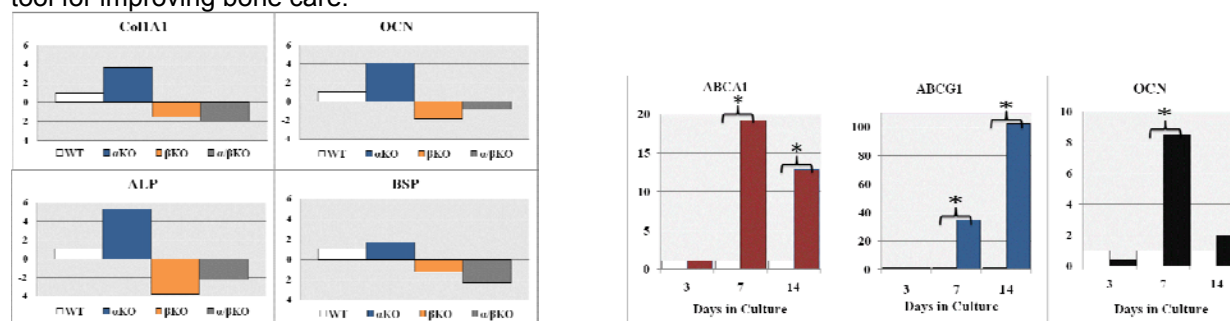


Fig.1: Fold-change in gene expression LXR KO and WT animals. Fig.2: Fold-change in OBs cultured with and without (white bars) DMHCA.

A NUCLEAR FUNCTION OF mRNA DECAPPING FACTORS IN WIDESPREAD PREMATURE

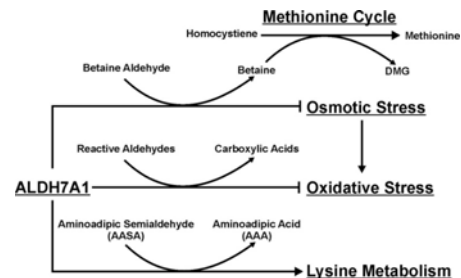
TERMINATION OF TRANSCRIPTION BY RNA POLYMERASE II. Kristopher Brannan, (PhD. MBP), Hyunmin Kim, Benjamin Erickson, Kira Glover-Cutter, Soojin Kim, Lauren Kiemele, Kirk Hansen, Richard Davis, Jens Lykke-Andersen, David L. Bentley Dept. Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO, 80045 Promoter proximal accumulation of RNA polymerase II (pol II) has recently been uncovered as a general feature of metazoan protein coding genes. Release of pol II from promoter proximal "pause" sites is now recognized as an important target for regulation of mammalian gene expression. We have found an unexpected role for transcription termination in regulating pol II elongation from promoter proximal regions. Immunoprecipitation and mass spectrometric analysis revealed that the nuclear 5'-3' RNA exonuclease Xrn2 associates with the ATPase termination factor TTF2 and the decapping factors Edc3, Dcp1a and Dcp2. Stable knockdown of these factors increased pol II occupancy both downstream and upstream of the transcription start sites (TSSs) at thousands of genes as determined by ChIP-Seq. Furthermore Xrn2, TTF2 and Dcp1a localize by ChIP-seq to many TSSs. These results suggest that premature termination mediated by a "torpedo" mechanism is a widespread feature of promoter-proximal transcription in human cells. This premature termination requires the nuclear activity of EDC3, Dcp1a and Dcp2, components of the mRNA decapping

complex previously thought to function exclusively in the cytoplasm. We propose that coupled pre-mRNA decapping and transcription termination are important for inhibiting productive pol II elongation, and that escape from premature termination may be a mechanism for up-regulating gene expression in development and disease.

BROCKER, C

CHARACTERIZATION OF ALDEHYDE DEHYDROGENASE 7A1 (ALDH7A1) AND THE POTENTIAL MECHANISMS UNDERLYING PYRIDOXINE-DEPENDENT EPILEPSY. C BROCKER (PHD, GS)¹, N LASSEN¹, D ORLICKY¹, K KAVANAGH², U OPPERMANN² AND V VASILIOU¹, ¹ UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS, AURORA, CO, USA AND THE ² STRUCTURAL GENOMICS CONSORTIUM, UNIVERSITY OF OXFORD, OXFORD, UK.

ALDEHYDE DEHYDROGENASES (ALDHS) CATALYZE THE NAD(P)⁺-DEPENDENT CONVERSION OF ALDEHYDES TO THEIR CORRESPONDING CARBOXYLIC ACIDS. MUTATIONS IN HUMAN ALDH7A1 ARE RESPONSIBLE FOR PYRIDOXINE-DEPENDENT EPILEPSY (PDE) AND FOLINIC ACID-RESPONSIVE SEIZURES. THE CONTINUING AIM OF THIS STUDY WAS TO CHARACTERIZE THE BIOCHEMICAL PROPERTIES AND FUNCTIONS OF ALDH7A1 IN AN EFFORT TO BETTER UNDERSTAND THE BROAD PHENOTYPIC SPECTRUM NOTED IN PDE PATIENTS. ALDH7A1 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS ATTENUATED HYPEROSMOTIC STRESS-INDUCED CELL DEATH CAUSED BY INCREASED SUCROSE OR SODIUM CHLORIDE (NACL). MOREOVER, CELLS WERE PROTECTED FROM TREATMENT WITH BOTH 4-HYDROXY-2-NONENAL (4-HNE) AND HYDROGEN PEROXIDE (H₂O₂). SIRNA KNOCKDOWN IN HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS INCREASED SUSCEPTIBILITY TO NACL-INDUCED HYPEROSMOTIC STRESS. TISSUE DISTRIBUTION STUDIES INDICATED HIGHEST EXPRESSION IN LIVER, KIDNEY AND BRAIN, FOLLOWED BY PANCREAS AND TESTES. SUBCELLULAR FRACTIONATION AND IMMUNOCHEMISTRY REVEALED LOCALIZATION WITHIN THE CYTOSOL, NUCLEUS AND MITOCHONDRIA, MAKING IT UNIQUE AMONG ALDHS. PURIFIED PROTEIN METABOLIZED A NUMBER OF ALDEHYDIC SUBSTRATES INCLUDING THE OSMOLYTE AND METHYL-GROUP DONOR PRECURSOR, BETAINE ALDEHYDE, LIPID PEROXIDATION-DERIVED ALDEHYDES AND THE LYSINE CATABOLITE, ALPHA-AMINOADIPIC SEMIALDEHYDE (AASA). HUMAN ALDH7A1 WAS CRYSTALLIZED AND STRUCTURALLY SUPPORTS ACCOMMODATION OF THE CHEMICALLY DIVERSE, EXPERIMENTALLY VERIFIED SUBSTRATE PANEL. MAPPING AND *IN SILICO* MUTAGENESIS OF HUMAN PDE MISSENSE MUTATIONS SUGGEST DIVERSE AND VARIABLE EFFECTS ON PROTEIN FUNCTION. IN CONCLUSION, ALDH7A1 IS A NOVEL ALDH EXPRESSED IN MULTIPLE TISSUES AND SUBCELLULAR COMPARTMENTS THAT APPEARS TO PLAY AN IMPORTANT ROLE IN SEVERAL, SEEMINGLY UNRELATED, BIOLOGICAL PROCESSES. [NIH SUPPORT: R01EY17963 (VV) AND F31AA018248 (CB)]



Castillo, D

PILOT STUDIES OF SONIC HEDGEHOG FUNCTION IN ADULT MOUSE LINGUAL EPITHELIUM D Castillo (Ph.D., GS)¹, K Seidel², O Klein², and LA Barlow¹ ¹Department of Cell and Developmental Biology and the Rocky Mountain Taste and Smell Center, University of Colorado School of Medicine, Denver, Colorado, United States of America, ²Department of Orofacial Sciences and Program in Craniofacial and Mesenchymal Biology, University of California San Francisco, San Francisco, California, United States of America. Abstract: The sonic hedgehog (Shh) pathway is involved in key regulatory events in embryogenesis, such as cell proliferation and differentiation. Postnatally it is involved in stem cell self-renewal, tissue maintenance and regeneration, while inappropriate activation of the Shh pathway has been implicated in multiple human cancers. Previous studies have demonstrated the importance of this signaling pathway for the embryonic development of taste buds in mammals, but its functional role in maintenance and self-renewal of adult taste buds is still elusive. Shh is expressed by a subset of cells within each taste bud, while its receptor Ptch and target gene, Gli1, are expressed by progenitor cells surrounding each bud, implying that Shh signals from within the bud to regulate taste bud progenitor cells. Here, we used an inducible, tissue specific Cre-lox system, in which K14creER, when induced, drives

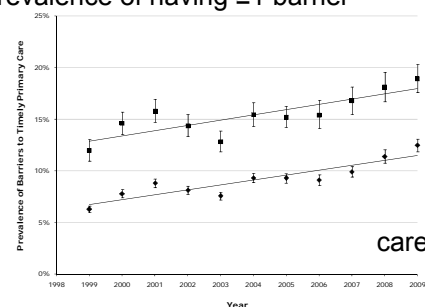
constitutive expression of Shh in a mosaic subset of taste and/or lingual epithelial cells that express the progenitor cell marker Keratin 14. When adult bigenic mice are given tamoxifen via oral gavage, Shh is upregulated in those cells where Cre has been activated. When mouse tongues were examined at 28 days after a single tamoxifen dose, we found, that Shh overexpression in the taste epithelium (epithelium surrounding taste buds) may trigger repression of taste bud formation, since our data show increase number of taste buds in control tongues when compared with Shh knocking tongues. Furthermore, we observed unexpected formation of ectopic taste buds in the general lingual epithelium; ectopic taste buds which comprise all 3 differentiated taste bud cell types. From these findings we infer that in adult mice, there is a differential competency to respond to Shh between the taste epithelium and the general lingual epithelium. Shh knocking in taste epithelium represses taste bud formation, while the lingual epithelium produces taste buds in response to focal overexpression of Shh, where in the absence of ectopic Shh, these taste buds never form. Our preliminary data in sum suggest the testable hypothesis that proper Shh pathway activity regulates proliferation and differentiation of taste buds in adult mice. Current studies are also underway to link Shh pathway function with that of other signaling pathways, such as Wnt, to uncover how adult taste buds are renewed and maintained.

Cheung, P

BARRIERS TO TIMELY PRIMARY CARE AND EMERGENCY DEPARTMENT UTILIZATION: IMPLICATIONS FOR HEALTHCARE REFORM

Paul T. Cheung, MS3, Candidate in the school of Medicine (presenting author); Jennifer L. Wiler, MD, MBA; Adit A. Ginde, MD, MPH; University of Colorado School of Medicine, Department of Emergency Medicine

Background: Patients are having increased difficulty in accessing primary care, and healthcare reform is expected to exacerbate this problem. Decreased primary care access may result in increased emergency department (ED) utilization. Objectives: To measure the prevalence and temporal trends in barriers to timely primary care and to determine the association between these barriers and ED utilization. Methods: We analyzed 317,497 adult participants of the 1999-2009 National Health Interview Survey (NHIS), an annual, nationally representative sample. Five specific barriers to timely primary care and the frequency of ED visits during the past 12 months were measured by self-report. We analyzed the survey-weighted data using multivariable logistic regression models for each barrier, adjusting for demographics, socioeconomic status, health conditions, and access to care. Results: Overall, 9.7% of adults per year had ≥ 1 barrier to timely primary care and 20.1% had ≥ 1 ED visit. Adults with a higher number of barriers were more likely to have ≥ 1 ED visit (18.8% for 0 barrier, 29.5% for 1 barrier, and 36.5% for ≥ 2 barriers). After adjusting for covariates, the following barriers were each independently associated with ≥ 1 ED visit: “couldn’t get through on phone”; (adjusted odds ratio [OR] 1.63; 95%CI, 1.52-1.74); “couldn’t get an appointment soon enough” (adjusted OR 1.53; 95%CI, 1.46-1.61); “waiting too long in doctor’s office” (adjusted OR 1.45; 95%CI, 1.38-1.52); “clinic not open when you could go” (adjusted OR 1.65; 95%CI, 1.55-1.75), “not having transportation” (adjusted OR 1.83; 95%CI, 1.70-1.98). The prevalence of having ≥ 1 barrier increased from 6.3% (95%CI, 6.0-6.6) in 1999 to 12.5% (95%CI, 11.9-13.1) in 2009. Concurrently, the prevalence of having ≥ 1 ED visit increased from 17.2% (95%CI, 16.6-17.7) to 21.2% (95%CI, 20.6-21.9). Among adults with ≥ 1 ED visit, the prevalence of having ≥ 1 barrier to timely primary care increased from 12.0% (95%CI, 11.0-13.0) to 18.9% (95%CI, 17.6-20.3). Conclusion: Barriers to timely primary care were associated with increased ED utilization. Over the past decade, multiple barriers to timely primary care have increased and were more prevalent among those with ED visits. These trends merit further investigation and may necessitate intervention as healthcare reform is implemented.



Inter- and intra-observer variation in measuring part-solid lung nodules using conventional and semi-automated methods Significance and background: Lung cancer remains the leading cause of cancer death for both women and men in the US. Majority of lung cancers are diagnosed at an advanced stage, with a dismal prognosis. Survival rates in lung cancer vary significantly by stage; with early diagnosis at stage (1A), the survival approach 70%, but with later diagnosis at stages IIA and IIIA, the survival rates fall dramatically to 34% and 13% respectively. These statistics underscore the importance of early detection of lung cancer. Low-dose CT scans is sensitive to detect lung nodules, potentially early stage cancer. Majority of these small nodules are benign. Growth of these small nodules is considered as surrogate biomarker for malignancy. But there are inherent limitations in accurate measurement of small nodules. Our study involves part-solid nodules as the rate of malignancy for part-solid nodules is higher than for pure ground glass and solid nodules (62.5 %, 19% and 7% respectively). Recent studies have shown that the solid component of part-solid nodules represent the invasive part of adenocarcinoma. It is believed that volumetric measurements of nodules are more accurate compared to longest diameter. There are few small studies which support this observation for solid nodules, but there is no previous study evaluating the part-solid nodules. Hypothesis: There will be greater variability in the measurement of part-solid nodules. Purpose: To assess the inter- and intra-reader variability in measurement of part-solid nodules using manual measurement on PACS and a semi-automated volumetric method. Methods: This retrospective study used low dose chest CT scans performed with similar technique from a lung cancer screening study. Five solid nodule and 5 part-solid nodule cases (one nodule/case) were chosen for this study. Three trained readers measured the nodules in a random fashion independently using two methods twice on the same day with preset breaks in the reading sessions. McKesson PACS system was used to measure the longest diameter (RECIST) in manual fashion. Siemens Oncology Workstation was used for semi-automated measurements of volume and longest diameter using a single seed-based algorithm / threshold for the entire part-solid nodule and for the solid portion. Results are yet to be analyzed.

HIGH BCL11B EXPRESSION IS CORRELATED WITH HIGH C-MYC EXPRESSION AND ANTI-APOPTOTIC PHENOTYPE IN MEDULLOBLASTOMA. BC Cristiano (MS II) 1, DK Birks 1, I Alimova 1, R Vibhakar 1, 2, (1) Department of Pediatrics, University of Colorado, Denver, CO (2) Children's Hospital Colorado, Aurora, CO. Medulloblastoma are a heterogeneous group of highly malignant brain tumors that most often affect children. Outcomes among children vary widely and C-MYC amplification is the single most important molecular indicator of poor prognosis. Here we analyzed gene expression in high MYC expressing tumors and compared these results to low MYC expressing controls to identify new therapeutic targets for high MYC expressing medulloblastoma. Primary tumor samples were obtained from surgeries performed at The Children's Hospital of Colorado. RNA extracted from these samples was profiled using whole genome microarrays. Gene expression from tumors with high MYC expression was compared to gene expression from low MYC expressing tumors. Differentially expressed genes were profiled using Ingenuity Systems pathway analysis software. The results of this analysis identified the leukemia associated zinc finger protein Bcl11b as a potential therapeutic target. Therefore, we pursued focused studies of its function in vitro. Medulloblastoma cell lines Daoy, UW228, D283 and ONS-76 were used for in vitro assays of malignancy. These assays included annexin V staining for apoptosis and colony focus assay, with or without silencing of BCL11B using short hairpin RNA expressing plasmids (shRNA). BCL11B is over expressed in both high MYC expressing primary tumor samples and high MYC expressing medulloblastoma cell lines. Furthermore, increasing MYC expression in Daoy by stable transfection with a MYC expressing plasmid increased BCL11B expression. Silencing of BCL11B expression produced a pro-apoptotic phenotype in Daoy and UW228 cells as measured by annexin V staining. Also, silencing of BCL11B expression blocked colony formation in both Daoy and ONS-76 as measured by colony focus assay. The leukemia associated zinc finger protein Bcl11b promotes an anti-apoptotic phenotype in medulloblastoma and may be important in the oncogenesis of high MYC expressing medulloblastoma. Thus, Bcl11b or its downstream effects represent potential therapeutic targets in high MYC expressing medulloblastoma.

DESCH, AN

EFFEROCYTIC CD103+ PULMONARY DENDRITIC CELLS PREFERENTIALLY ACQUIRE AND PRESENT APOPTOTIC CELL-ASSOCIATED ANTIGEN. A. Nicole Desch² (PhD, GS), Gwendalyn J. Randolph⁴, Kenneth Murphy⁵, Emmanuel Gautier⁴, Ross Kedl^{2,3}, Mireille H. Lahoud^{6,7}, Irina Caminschi⁶, Ken Shortman^{6,7}, Peter M. Henson^{1,2}, and Claudia V. Jakubzick^{1,2} ¹Department of Pediatrics, ²Integrated Department of Immunology, ³Department of Medicine, National Jewish Health and UC Denver Anschutz Campus, Denver, CO ⁴Department of Gene and Cell Medicine, Mount Sinai School of Medicine, NY, NY ⁵Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO ⁶The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia ⁷Department of Medical Biology, The University of Melbourne, Melbourne, Australia. Cells undergoing programmed cell death (apoptosis) are removed in situ by macrophages and dendritic cells through a specialized form of phagocytosis (efferocytosis). In the lung there are two primary dendritic cell (DC) subsets with the potential to migrate to the local lymph nodes and initiate adaptive immune responses. Here we show that only CD103+ DCs were able to acquire and transport apoptotic cells to the draining lymph nodes (LNs) and cross present apoptotic cell-associated antigen to CD8 T cells. In contrast, both the CD11bhi and the CD103+ DCs were able to ingest and traffic latex beads or soluble antigen. CD103+ DCs selectively exhibited high expression of TLR3 and ligation of this receptor led to enhanced cytotoxic T cell responses in vivo to apoptotic cell-associated antigen. The selective role for CD103+ DCs was confirmed by studies in Batf3^{-/-} mice, which lack this DC subtype. Our findings lead to the conclusion that CD103+ DCs are the DC subset in the lung that captures and presents apoptotic cell-associated antigen under homeostatic and inflammatory conditions and raise the possibility for more focused immunologic targeting to CD8 T cell responses.

DRASIN, D

MicroRNA-424 as a potential regulator of mammary cell epithelial-to-mesenchymal transition and breast cancer stem-like cell properties The epithelial-to-mesenchymal transition (EMT) is a cellular program in which epithelial cells lose epithelial characteristics and gain mesenchymal characteristics, resulting in a range of acquired traits such as increased plasticity that allow stationary epithelial cells to become motile. This process is necessary for proper development during embryogenesis and for wound healing later in life. A similar aberrant, and more transient, EMT program can contribute to the metastatic dissemination of epithelial cancer cells, and is referred to as an oncogenic EMT. Recent breast cancer studies have identified components of the microRNA-200 family as major players in maintaining the epithelial phenotype of cancer cells; the loss of the microRNA-200 family coincides with more aggressive breast cancers in humans. We find that through the course of an induced EMT in human mammary cells, mesenchymal programming is activated prior to epithelial gene repression and prior to loss of members of the microRNA-200 family; however, epithelial programming ultimately decreases greatly. This simultaneous expression of mesenchymal and epithelial proteins at this early time point, as opposed to an all or none phenotype, more closely resembles changes seen in many human carcinomas. To identify microRNAs important during this early mesenchymal-like transition, we performed a microRNA microarray screen. We identified multiple oncogenic EMT inducers as up-regulating microRNA-424 concomitantly with the induction of a mesenchymal-like phenotype, and before the repression of epithelial markers and the microRNA-200 family. Overexpression of microRNA-424 in non-transformed human mammary epithelial cells is sufficient to produce a mesenchymal-like phenotype, as well as increase the mammary stem cell population in vitro. These data suggest that microRNA-424 may play an important role in both the mesenchymal programming and increased tumor-initiating cell formation that occur during an oncogenic EMT. Further investigation of microRNA-424 may yield new targets for inhibiting EMT and/or tumor-initiating cell formation during breast cancer.

Elkasrawy, Moataz (Newman) **EXPERIMENTAL BPA-FREE ORTHODONTIC BRACKET ADHESIVE: BOND STRENGTH.** M Elkasrawy (DDS, SODM), SM Newman, Department of Restorative Dentistry, School of Dental Medicine

Light cured composite bonding systems have been successfully used to bond orthodontic brackets to teeth. Despite the fact the BPA content or from degradation is well below any scientifically supported concentrations that should be of concern, the increased public awareness of the potential dangers of bisphenol A (BPA), that might be released from dental products has prompted researchers to seek alternatives that offer greater piece of mind. **Objective:** Investigate experimental BPA-free formulae as orthodontic bracket bonding systems for bond strength. **Methods:** The materials examined were two experimental BPA-free formulas (Septodont), Transbond XT (3M/Unitek), Illuminate (Ortho Organizers), Light bond (Reliance), and Resilience (OrthoTech). Brackets (MiniTwin) were bonded by normal bracket bonding techniques to bovine enamel flattened to 600 grit smoothness. The etchant was 37% phosphoric acid for 30 seconds. The primer resin of each formulation was applied in a thin coat. The adhesive was applied to the bracket and the bracket pushed onto the tooth. The primer and adhesive was light cured for 60 seconds with a dental curing light. The bonded teeth were mounted with gypsum in a delrin test mold using an orienting jig. The specimens were stored wet at 37°C for 1 week. The bonds were stressed in the shear mode and the amount of adhesive remaining on the teeth evaluated on an ordinal scale. **Results:** BPA-free2 (18.1±4.5MPa), Tansbond (19.7±3.4), and Light Bond (17.3±4.6) gave the highest bond strengths and were not significantly different (ANOVA with SNK , p≤0.05). Resilience (14.4±3.4), BPA-free1 (13.4±3.4), and Illuminate (11.9±3.3) had lower strengths not statistically distinguishable. The adhesive remaining index had statistical differences among all of the samples with Transbond leaving the most adhesive>BPA-free2>BPA-free1>Resilience>Light Bond>Illuminate (Kruskal-Wallis and SNK, p≤0.05). **Conclusions:** Both experimental formulae are commercially acceptable with the BPA-free2 among the strongest for effective bracket retention during orthodontic treatment. Such formulations offer alternative bonding systems that alleviate all concerns of BPA exposure, despite the fact that the exposure from dental materials with current formulae is inconsequential.

FERTILITY PRESERVATION ATTITUDES AND PRACTICE SURVEY ADMINISTERED TO ONCOLOGY PROVIDERS AT THE UNIVERSITY OF COLORADO DENVER.

Objective: To determine attitudes and practice regarding fertility preservation (FP) for reproductive age oncology patients among a group of oncology providers. **Design:** Cross sectional survey of University of Colorado (UCH) oncology providers regarding attitudes and practices relating to FP. **Materials and Methods:** A FP survey was created using the web-based survey tool, Zoomerang and administered via email to 252 oncology providers identified through oncology specific listserves created within the UCH Information Technology department. Providers included physicians, nurses, social workers, pharmacists, and nurse practitioners. The study aimed to determine attitudes and practices regarding FP options, and to ascertain provider preferences regarding FP counseling of patients. The survey was active for 4 weeks, 2 reminders were sent for completion. Data were extracted and analyzed using descriptive analysis. **Results:** The response rate was 30%, the rate for MDs was 23% (20 of 86). Of all respondents only 11% felt well informed regarding FP options. Among MD's, 50% of responders (n=10) felt that FP was an important issue, and 45% (n=9) reported routinely discussing FP with patients. Of the 19 total responders (MDs plus others) who routinely discuss FP, only 4 of them felt well informed about the options at UC and only 21% reported referring patients to specialists in reproductive medicine. 78% of all responders felt that oncologists should initiate the discussion of FP with patients, and further education would best be provided by either referral to a reproductive specialist, informational website, or providing comprehensive written information. **Conclusions:** Oncology providers at UCH do not feel well informed regarding FP, and are generally unaware about available options. While this is an important issue to many providers, few discuss FP with their patients or refer them to a reproductive specialist for consultation. To increase awareness regarding FP for reproductive age oncology patients, there must be increased collaboration among oncology providers and reproductive specialists.

CHARACTERIZATION OF PHAGOCYtic MAMMARY EPITHELIAL CELLS DURING POSTPARTUM INVOLUTION. J Fornetti, (Ph.D., GS), P Henson, V Borges, and P Schedin, Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO. Women with breast cancer diagnosed postpartum have a poorer prognosis than patients without a recent pregnancy. Our lab has identified postpartum mammary gland involution as a normal, physiologic process following pregnancy and lactation that is tumor-promotional. During involution, 50-80% of the milk-producing mammary epithelial cells (MECs) die. Recent data indicate that the MECs themselves become phagocytic and clear the gland of their dead neighbors. Peak phagocytosis precedes an influx of M2 macrophages that likely contribute to a pro-tumorigenic microenvironment. The goals of this work are to further characterize phagocytic MECs during involution and test the hypothesis that phagocytic MECs secrete cytokines that promote M2 macrophages. To study phagocytic MECs in vitro we developed a model in which murine mammary epithelial cell monolayers are treated with TGF- β 3, a putative inducer of postpartum mammary gland involution in vivo. In this model, TGF- β 3 leads to disruption of cell junctions and induction of phagocytosis. We hypothesize MEC junction disruption is necessary for the cells to become phagocytic. Supportive of this hypothesis, in vivo IHC data demonstrate TGF- β signaling in MECs at the onset of involution, and cleavage and reorganization of E-cadherin corresponding with the time at which cells become phagocytic. Experiments are underway to further explore the role of MEC junction disruption in phagocytosis. To address the question of whether phagocytic MECs promote M2 macrophages, bone marrow-derived macrophages (BMDMs) are treated with conditioned media from phagocytic MECs and analyzed by western blot and qPCR for markers of M1 and M2 macrophage activation. Data to date are consistent with phagocytic MECs promoting immunosuppressive M2-like macrophages, though additional work needs to be done to further characterize the macrophage phenotype. We anticipate this work will elucidate the role of phagocytic MECs in regulating the immune microenvironment in the postpartum involuting mammary gland, and lead to the identification of new targets for postpartum breast cancer prevention and therapy.

NOVEL BPA-FREE MONOMER FOR DENTAL APPLICATIONS. L Grow (DDS, SODM), JW Stansbury, SM Newman, Department of Restorative Dentistry, School of Dental Medicine

A new, high MW, non-BPA monomer (FIT852, Esstech) is being proposed as a high conversion, low shrink resin. Other high MW non-BPA monomers can improve copolymer properties Objective: This study investigates the properties of this monomer and its homopolymer, as well as several other monomers and the resulting copolymers. . Methods: 0.1% DMPA was added to each of the following homomonomers: BisGMA, UDMA, CN992, CN999 (Sartomer) and FIT852,. The conversion kinetics was measured with peak area at 6170 cm^{-1} in near-IR. An Acticure curing light was used with a 365 nm filter with irradiances of 500 mW/cm^2 for 10 min or 1 min, and 100 mW/cm^2 for 10min (N=3). The flexural properties were measured dry (N=5) after 1 week at 37°C on $2\text{X}2\text{X}20$ mm span beams on a mechanical testing machine at 1 mm/min. Another set was stored in water until weight was stable and flexural properties measured. Polymerization shrinkage was measured on an ACTA linometer at 10 min under 500 mW/cm^2 . Water sorption, vinyl concentration, and refractive index were also determined. Results: Specimens of FIT852 and CN992 did not break and peak stress was reported. Comonomers were studied for the CN and FIT852 resins.

	Conversion	Flexural Strength (Mpa)		Flexural Modulus (Gpa)		Polymerization Shrinkage (%)
		DRY	WET	DRY	WET	
FIT852	0.892 \pm 0.003	53.7 \pm 7.5	6.9 \pm 2.4*	1.25 \pm 0.08	0.05 \pm 0.03	2.25 \pm 0.49
BisGMA	0.473 \pm 0.023	127.6 \pm 8.9	15.6 \pm 3.6	2.79 \pm 0.20	0.22 \pm 0.04	2.41 \pm 0.08
UDMA	0.746 \pm 0.005	79.1 \pm 18.1	41.6 \pm 24.4	2.13 \pm 0.12	1.13 \pm 0.30	4.80 \pm 0.08
CN999	0.869 \pm 0.08	169.29 \pm 12.1		3.872 \pm 0.11		FIT852/CN999- 7.53
CN992	0.999 \pm .03	3.347 \pm 1.03*	*peak stress	0.022 \pm 0.008		FIT852/CN992- 8.23

Conclusions: The FIT852, BPA-free, homomonomer achieves high conversion with low polymerization shrinkage. The polymeric strength shows low peak stress and low modulus, with much lower values when wet. This monomer could prove useful as a substitute for BisGMA in composite resin formulations. The copolymer CN999/FIT852 has high strength but not low shrinkage.

HAO, Y

BIOPHYSICAL ANALYSIS OF THE SELF-ASSOCIATION OF BACTERIOPHAGE PHI29 FAMILY PACKAGING RNAS. Y Hao, (Ph.D., GS), JS Kieft, Molecular Biology Program, School of Medicine, University of Colorado, Denver, CO During viral assembly, the phi29 bacteriophages must package their 19.3 kilobase-long DNA genome into the capsid. They do this using a powerful packaging motor that contains three components: a connector protein, a packaging RNA (pRNA) and an ATPase. This RNA-protein, ATP-dependent motor packages the genome in less than five minutes and the packaging process is under tremendous back-pressure (~79 piconewton). So far, the phi29 family of bacteriophage is the only one known to use a pRNA in this process, and the pRNA is the only RNA reported that forms a higher-order multimer by intermolecular “kissing” interactions of identical molecules that are essential for the function of the motor. This self-association property gives the pRNA great potential as a building block in nanotechnology. The pRNA is required for DNA packaging, but its precise role in the whole packaging motor is unknown. To gain insight into the role of the pRNA, we are studying the pRNAs from different members of the phi29 family strains, which have different sequences and kissing interactions. Using a combination of biochemical and biophysical methods to include native gel electrophoresis, dimethyl sulfate (DMS) probing, and analytical ultracentrifugation (AUC), we are exploring the conformational dynamics within the pRNA, the relationship of these dynamics with multimer formation, and the different abilities of these pRNAs to self-associate. We have discovered that the length and sequence in the kissing interaction region plays an important role in multimer formation dynamics and stability. Using this information, we are developing new testable hypothesis for how these RNAs self-associate and how this relates to binding to the other components of the motor. Ultimately, these insights information for pRNA will greatly help us understand pRNA role in the packaging motor and further engineer for nanotechnology.

HUDAK, M

POSITIVE DEVIANCE IN ACUTE ISCHEMIC STROKE CARE. ML Hudak (MD, MSII), AM Graves, KA Reichelt, JR Sweigart, EM Harry, JJ Glasheen, W Jones, and EU Cumbler, University of Colorado School of Medicine and Hospital, Aurora, Colorado. As of July 31, 2011, only 133 national stroke centers have been recognized on the American Heart Association (AHA) Target: Stroke Honor Roll. At these hospitals, at least 50% of treated ischemic stroke patients receive IV thrombolysis within an hour of arrival. Positive deviance methodology examines a common challenge and identifies successful outliers. Structured phone interviews were conducted with Stroke Program Coordinators of 12 Honor Roll centers. Detailed descriptions of evaluation and treatment process and methodology were elicited. Emphasis was placed on changes instituted, barriers, and delays. Utilization of the 10 AHA Target: Stroke best practice strategies was assessed. Additional techniques and aspects of institutional culture were identified. Seven of ten AHA best practice strategies were employed by 100% of the interviewed hospitals. However, three strategies were utilized by only 50-60% of the hospitals. Additional techniques identified included: active time monitoring, allowing t-PA order with lab results pending, utilization of non-Neurologist staff as lead in stroke alerts, and visual performance feedback to staff. Common themes of institutional culture included strong stroke champions in multiple departments, empowering staff to find department-specific solutions to solve the common challenge, and setting progressively more ambitious targets. Although the majority of the AHA strategies were supported by the interviewed hospitals, many centers modified methods to achieve the goal of Target: Stroke. For rapid treatment of ischemic stroke the “positive deviance” lies in institutional culture of urgency and continuous improvement. Best practice hospitals create a healthy competition amongst staff, solidify interdepartmental partnerships, instill an equal sense of accountability, open communication, and an atmosphere of teamwork. A great deal of variation was discovered in roles and processes employed by the hospitals, though all identified the essential goal of fostering a culture of motivation and urgency.

HUDISH, T
OPINCARIU, L

N-NITROSO-TRIS-CHLOROETHYLUREA INDUCES PRE-MALIGNANT SQUAMOUS DYSPLASIA IN MICE TM Hudish^{1,2†}, (M.D., SOM), LI Opincariu^{1,2†}, AB Mozer¹, MS Johnson¹, TG Cleaver², SP Malkoski², DT Merrick¹, and RL Keith^{1,2,3} ¹Denver Veterans Affairs Medical Center, Department of Medicine, Denver, CO ²University of Colorado Denver, Department of Medicine, Aurora, CO ³Faculty Sponsor †These authors contributed equally to this work Purpose: Squamous cell carcinoma (SCC) and pre-malignant endobronchial lesions have been difficult to study in murine models. In this report, we evaluate the topical N-nitroso-tris-chloroethylurea (NTCU) murine SCC model, determine the extent to which resulting pre-malignant airway dysplasia develops, discuss clinicopathologic grading criteria in lesion progression, and confirm that immunohistochemical (IHC) staining patterns are consistent with those observed in human endobronchial dysplasia and SCC. Methods: Male and female FVB mice were treated biweekly with topical NTCU (4, 8, or 40mM) or vehicle for 32 weeks. Following sacrifice, squamous cell lesions were enumerated and categorized into the following groups: flat atypia, low-grade dysplasia, high-grade dysplasia, and invasive SCC. Results: The 40mM NTCU concentration produced the entire spectrum of premalignant dysplasias and squamous cell carcinomas, but was associated with poor survival. Concentrations of 4mM and 8mM NTCU were better tolerated and produced significant levels of only flat atypia. Squamous origin of the range of observed lesions was confirmed with IHC staining for cytokeratin 5/6, p63, thyroid transcription factor-1 (TTF-1), and Napsin-A. Conclusion: This study demonstrates that topical application of high dose NTCU produces endobronchial pre-malignant lesions with classic squamous characteristics and should allow for improved pre-clinical evaluation of potential chemopreventive agents.

ILLING, D

P-SELECTIN LOCALIZATION IN PLATELET AGGREGATES FORMED UNDER FLOW. Iling, D.A.R. (MD, SOM), Brodsky, G. L., DiPaola, J., Department of Pediatrics, University of Colorado, Aurora, CO The real-time localization of P-selectin in platelets formed under flow on a fibrillar collagen substrate was investigated. Experiments were conducted using a microfluidic device, developed previously for analysis of platelet phenotypes under flow. The formation of platelet aggregates was observed in real-time using fluorescence microscopy with anti-CD41 and anti-P-selectin fluorescent antibodies. After blood was drawn into CTI/Citrate tubes, it was incubated with the fluorescent antibodies, and then recalcified with CaCl₂ and added to the microfluidic device. Sufficient CaCl₂ was added to increase the final blood [Ca⁺⁺] by 2 or 20 mM, depending on experimental aim; final blood [Ca⁺⁺] is not directly known due to lingering Ca⁺⁺ chelator effects. The blood was drawn through the device, mounted on a glass slide with a strip of deposited fibrillar collagen. The slide with collagen was incubated with bovine serum albumin before the experiment to prevent activation of platelets by the slide. The blood wall shear rate in the device was a constant 100 1/s, representing conditions found in the body. In select experiments, PPACK (D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone), a direct thrombin inhibitor, and abciximab, a GP IIb/IIIa inhibitor, were added to blood to assess differential effects of activation pathways on P-selectin localization. Platelet aggregates formed under higher [Ca⁺⁺] resulted in small, string-like, aggregates with diffuse P-selectin expression throughout the aggregate in a pattern similar to a honeycomb. Platelet aggregates formed under lower [Ca⁺⁺] were large, often rounded, and displayed P-selectin localization to the periphery of the forming aggregates; large P-selectin devoid areas were seen inside the aggregate. In low [Ca⁺⁺] experiments, the addition of PPACK did not result in any significant changes in aggregate morphology or P-selectin localization. Addition of abciximab to the low [Ca⁺⁺] experiment resulted in diffuse localization of P-selectin throughout the aggregate, similar to the results in the high [Ca⁺⁺] case. The results from these experiments indicate that platelet aggregate morphology and P-selectin localization is dependent on extracellular calcium concentration and GP IIb/IIIa signaling.

JACK, J

Long-term Neuropsychological Outcomes after Childhood Arterial Ischemic Stroke (AIS) Jessica Jack, BA, Richard Boada, PhD Neil A. Goldenberg, MD, PhD, Marilyn Manco-Johnson, MD, and Timothy J. Bernard, MD Background: Childhood AIS affects 2-6/100,000 children per year and is associated with significant morbidity and mortality. Although previous reports have demonstrated lower IQ's in children following AIS as compared to healthy children, understanding of cognitive function more broadly in childhood AIS remains limited. Methods: In order to address this gap in knowledge, we collected data on demographics, stroke type, stroke size, stroke location, and long-term neuropsychological outcome. Patients were enrolled in an institution-based prospective cohort study of childhood AIS, comprising 63 children diagnosed with AIS from March 2006-August 2011. Neuropsychological assessment employed age-appropriate measures. In addition to IQ, other cognitive domains were tested, including academics, visual-motor skills, memory, and language. Results: 42 patients in our cohort had neuropsychological testing available at least 10 months after stroke diagnosis: 21 males and 21 females, with a mean age of 7 years at time of stroke (SD= +/-5.43). Mean neuropsychological testing was at 36 months after AIS (SD= +/- 30.72). Mean IQ for childhood AIS patients was 93.8 (SD= +/-17.75) and was significantly lower than the population mean ($p < .05$). Cognitive profile testing revealed significantly decreased functioning in the visual-motor (mean standard score = 84.1 [SD = +/-17.32], p

JOHNSON, C

Excimer laser surgery for the correction of refractive errors is becoming one of the most widely performed ocular procedures. The surgery involves ablation of the corneal stroma and, in some cases, the corneal epithelium. Concern has increased about the potential health risks to ophthalmologists performing photorefractive keratectomy and laser in situ keratomileusis, more commonly referred to as Lasik surgery. Previous work found that excimer laser plumes of smoke generated from Lasik surgery contain respirable particles of roughly 0.22 μm in diameter, capable of transmitting live virus. The purpose of this study is to investigate the cytokine response of mouse and human cells exposed to plumes of smoke generated from Lasik-ablated corneal tissue in culture. Plumes were generated from intact adult bovine eyes using one series of ten PTK procedures and filtered through media under vacuum. RAW264.7 cells were exposed to the following complete media conditions in triplicate: unexposed, plume-filtered, air-filtered, and lipopolysaccharide. To quantify various cytokines in cell culture media, supernatant collected at various time points was analyzed using ELISA. Media containing plume particles did not induce a statistically significant release of IL-6 and TNF α in exposed RAW264.7 cells as compared to negative controls ($p > 0.05$). Currently, the cytokine response of mouse and human pulmonary cells exposed to Lasik plume particles is being assessed. We expect to identify significantly higher levels of cytokines released from mouse and human pulmonary cells after repeated exposure to Lasik plume particles as compared to negative controls.

LEASIA, K

Periventricular Leukomalacia (PVL) is the most common ischemic brain injury to white matter in prematurely born infants. Developmentally, oligodendrocyte precursor cells (OPCs) become the white matter of the brain, wrapping axons as myelin. PVL causes cerebral palsy in 60-100% of infants and the incidence of PVL ranges from 4-26% in neonatal intensive care units. At autopsy, as much as 75% of premature infants show PVL. Cerebral palsy has an array of manifestations including spastic diplegia, quadriplegia, blindness, intellectual and/or developmental impairment. The purpose of this study is to investigate the underlying mechanism of PVL with a focus on OPC survival and how hypoxic conditions, such as those seen in premature birth, may affect this. A hypoxic environment during OPC development uncouples respiration to ATP production. Furthermore, hypoxic conditions result in higher amounts of naturally occurring reactive oxygen species (ROS). OPCs are particularly vulnerable to ROS because they have a lower level of superoxide dismutase, an enzyme that combats these reactive oxygen derivatives. Similarly, in any stressed environment such as low oxygen one, inflammation can occur leading to collateral damage of surrounding tissue. We hypothesize that a low oxygen environment may interfere with OPC survival, specifically resulting in either no OPC development, OPC development without specification, or OPC development with subsequent death. Once OPCs are rendered dysfunctional, myelin wrapping of axons is abnormal and PVL will occur. The methods employed in this study are simulating hypoxic conditions in zebrafish embryos (*Danio rerio*) by applying certain inhibitors to

oxidative phosphorylation. The experiment focuses on different inhibitor concentrations at different times in development during a window of vulnerability for OPC specification in the neural tube. The goal of this study is to gain an insight into the mechanism behind PVL in the context of oxygen deprivation in utero.

LIM, D

CLONING AND CHARACTERIZATION OF AGXT (ALANINE GLYOXYLATE AMINOTRANSFERASE)

GENE PROMOTER. DD Lim, (M.D., SOM), HK Koul, PhD, Department of Urology, University of Colorado, Denver, CO. Primary hyperoxaluria type 1 is a rare autosomal-recessive disorder caused by abnormal activity of the liver specific enzyme, peroxisomal alanine glyoxylate aminotransferase (AGT). The AGT enzyme acts as a catalyst in the transamination (detoxification) of glyoxylate to glycine. Without functioning AGT, glyoxylate is converted into oxalate. Oxalate is renally excreted and in high concentrations will form insoluble calcium salts that deposit into the kidneys as well as other tissues. With an AGT deficiency, nephrolithiasis and/or nephrocalcinosis (renal parenchymal calcification) can occur resulting in oxaluria (extra-renal oxalate deposition) and end-stage renal disease (ESRD). When kidneys are compromised, plasma oxalate levels dramatically increase leading to the deposition of oxalate in many body tissues with severe consequences. Dialysis often fails to control the hyperoxalemia and these patients eventually die. Presently there is no corrective therapy available to PH-1 patients. Even in patients that receive renal transplantation, outcomes are poor as the transplanted kidneys are rapidly damaged by the high oxalate load. At present, a combined liver and kidney transplant are needed for these patients, but quality of life as well as survival are poor. Yet, despite the poor outcome, only small progress has been made towards toward effective treatments. The goal of this study is to understand how the AGT gene promoter is regulated by generating fragmented AGT-Luciferase plasmid constructs and analyze their relative transcriptional contribution. This method will be used to identify critical transcription factors and their binding sites within the AGXT promoter which are involved in AGXT regulation. Lastly, by understanding how the AGXT promoter is regulated, these studies may help identify subsets of patients with promoter dysfunction and may lead to future therapeutic strategies.

MANN, S

BIOMECHANICAL BASIS FOR RESISTANCE EXERCISE PRESCRIPTION FOR A PERSON 40 YEARS AFTER TRAUMATIC UPPER EXTREMITY AMPUTATION – CASE REPORT

SRE Mann (DPT, School of Medicine Physical Therapy Department), C Christiansen (PT, PhD., Faculty School of Medicine Physical Therapy Department) Background and purpose: This case report highlights the effectiveness of applying biomechanical principles to develop high-level resistance exercise programs for healthy people with chronic upper extremity amputation. Guidelines for resistance exercise prescription are needed for people with chronic upper extremity amputation. Exercise recommendations after upper extremity amputation focus primarily on early management, concluding after the patient has learned successful prosthetic operation and reintegrated into the community. Methods: A 62-year-old male with a chronic (40-year) left transradial upper extremity amputation and bilateral shoulder pain completed six weeks of an individualized resistance exercise program. The exercise program emphasized application of biomechanical principles, advanced exercise-specific weight training, and neuromuscular reeducation. The program goals included: maximized biomechanical efficiency during weight training activities, decreased shoulder pain with joint preservation, increased upper extremity and trunk muscle symmetry, increased functional range of motion in the cervical spine, and improved upper extremity function. Results: Goals met included: improved resistance exercise performance as assessed by magnitude of weight lifted, observational task analysis, and the Patient Specific Functional Scale; decreased shoulder pain as measured by patient report; improved upper extremity and trunk muscular symmetry as measured by manual strength testing using a hand-held dynamometer; improved cervical range of motion; and improved upper extremity function based on the Patient Specific Functional Scale and the QuickDASH questionnaires.

MARTIN, T

CONNEXIN 35 IN ZEBRAFISH SPINAL CORD DEVELOPMENT. TC Martin (M.D./Ph.D., GS), and AB Ribera, Ph.D., Department of Physiology and Biophysics, Neuroscience Program, Colorado Clinical and Translational Sciences Institute, University of Colorado Anschutz Medical Campus. Vertebrate connexin proteins form the majority of gap junction channels allowing direct communication between coupled cells. In both neuronal and non-neuronal tissues, many connexin proteins display strong embryonic expression and are subsequently downregulated, which raises the possibility these proteins may play developmental roles. For example, a number of developmental diseases in cardiac and epidermal tissue have been linked to mutations in connexin protein genes. Moreover, the involvement of gap junction proteins in neurodevelopmental processes has recently become evident, but the underlying mechanisms by which these proteins may mediate developmental processes remain poorly understood. This project aims to explore the role of a specific connexin (connexin 35, Cx35) in spinal cord development. Current evidence on the connexin gamma subfamily, of which Cx35 is a member, overwhelmingly supports neuronal specific expression. We are using the zebrafish embryo to uncover the developmental processes requiring Cx35. This model allows for easy genetic manipulation with morpholino knockdown technology. Additionally, the availability and ability to develop transgenic lines that express fluorescent markers under the control of cell-specific promoters allow for reliable identification of different cell types. RNA expression data indicate cx35 expression begins at 50% epiboly by RT-PCR and is localized to the spinal cord with RNA in situ hybridization at 24 hpf. Expression of cx35 is preferentially ventral in the spinal cord across all stages studied. The results of preliminary Cx35 morpholino knockdown suggest a requirement for this connexin protein in development of a subset of spinal cord cells. In order to identify the mechanistic basis for this role of Cx35, we are first identifying the specific cell types that express Cx35 across relevant developmental stages and whether these are the cells that develop abnormally upon knockdown of Cx35. Next, we aim to determine the networks of electrically coupled cells that depend on Cx35 function and how these networks change during development. The results of these studies will provide insights into developmental mechanisms that require Cx35.

MARTINEZ, E
MCABEE, K

MINING THE COLORECTAL CANCER SCREENING NETWORK TO EXPLORE PRACTICES, POLICIES, AND CHALLENGES IN COLORECTAL CANCER SCREENING. E. Martinez, K. McAbee, (MPH, CSPH) and HJ Wolf (PhD, MSPH), Colorado School of Public Health, Aurora, CO. Background: Colorectal cancer (CRC) is the second-leading cause of cancer-related deaths in the U.S. Achieving higher rates of CRC screening is needed to prevent and detect cancer. Several factors, including policies and practices, contribute to a program's ability to achieve a higher screening rate. Objectives: To survey the National Colorectal Cancer Screening Network about practices, policies and challenges associated with CRC screening. Methods: A literature review, preliminary survey (N=85) and key informant interviews were used to develop a detailed survey. The detailed survey was sent out electronically to a convenience sample Network members. Results (N=41) were analyzed with descriptive statistics and qualitative methods. Results: Overall, participants had strong awareness of key issues, ie, a screening colonoscopy being classified as diagnostic when a polyp is found and the resulting cost-sharing. Participants also reported knowledge of providers limiting the number of Medicare and Medicaid patients, largely due to reimbursement issues. Furthermore, many states had worksite wellness efforts to help increase screening rates. Conclusions: Several areas for CRC screening were reinforced. Recommendations for action include: 1) Address classification of screening colonoscopies; 2) expand worksite wellness policies; 3) train people in policy; and 4) implement additional surveys with healthcare providers.

METTLER, L

WHITE MATTER INTEGRITY IN INSULA AND FORNIX IMPLICATED IN BULIMIA NERVOSA

NEUROBIOLOGY. Lisa N. Mettler B.S. a,c , Megan E. Shott B.S.a, Michael D.H. Rollin M.D.b, Guido K.W. Frank M.D.a,b a Department of Psychiatry, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA, b Neuroscience Program, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA, c University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO, USA. This study investigated brain white matter (WM) functionality in bulimia nervosa (BN) in order to better characterize brain function in this disorder. Twenty-one control women (CW, mean age 27±7 years) and 19 women with BN (mean age 25±5 years) underwent diffusion tensor imaging (DTI) of the brain to calculate fractional anisotropy (FA; giving an indication of WM axon integrity) and the apparent diffusion coefficient (ADC; reflecting WM cell damage). Insula and fornix FA were significantly reduced in BN and FA values in those regions were negatively correlated with state and trait anxiety in CW but not BN. ADC values were increased in BN in the fornix, frontal WM regions, and the superior longitudinal fasciculus. BN ADC values were positively related to bulimia symptoms and adverse childhood events. WM integrity is disturbed in BN, and fornix and insula WM axon abnormalities are particularly implicated in BN, as previously reported in anorexia nervosa. Bulimic behavior and adverse childhood life events seem to be directly related to WM cell break down in BN.

MOSER, E

EFFECT OF DIPEPTIDYL PEPTIDASE-IV INHIBITOR TREATMENT ON POST-PRANDIAL GLUCAGON AND GLUCAGON-LIKE PEPTIDE-1 LEVELS IN PATIENTS WITH TYPE 1 DIABETES

EG Moser (M.D., SOM), JK Snell-Bergeon, SK Garg Barbara Davis Center for Diabetes, University of Colorado Anschutz Medical Center, Aurora, CO Peripheral insulin resistance in type 1 diabetes may be related to increasing body mass index (BMI), subcutaneous insulin delivery and a small, but significant, paradoxical rise in glucagon after meals. Dipeptidyl peptidase-IV (DPP-IV) inhibitors increase glucagon-like peptide-1 (GLP-1) resulting in a decrease in the paradoxical post-prandial rise of glucagon. This study evaluated the effects of sitagliptin, a DPP-IV inhibitor approved for patients with type 2 diabetes, in adult patients with type 1 diabetes. Our previous small pilot study evaluating the use of sitagliptin in patients with type 1 diabetes showed a statistically significant improvement in glucose control, insulin dose and A1c while patients were taking sitagliptin. This investigator-initiated, multi-center, double-blind, randomized, parallel, 20-week study enrolled 141 adult subjects with type 1 diabetes. The primary outcome was post-meal reduction in 4-hour glucagon area under the curve (AUC) following a meal challenge test with Boost™. Subjects received sitagliptin 100 mg/day or matching placebo for the 16-week study period following a 4-week run-in phase. A subset of 100 patients wore a blinded continuous glucose monitor (CGM) for 5 separate 7-day periods during the study. Secondary endpoints included changes in A1c, insulin dose, weight, hypoglycemia and GLP-1 and glucose-dependent insulinotropic peptide (GIP) levels and CGM data analysis. The baseline characteristics were similar between the two groups. The results from this trial are currently being analyzed. We predict that sitagliptin use in type 1 diabetes will improve glucose control (A1c) with a potential reduction in insulin dose or weight by decreasing the paradoxical rise in glucagon after meals. We further predict that the drug will be effective in increasing post-meal GLP-1 and GIP levels, similar to what occurs in patients with type 2 diabetes.

MURAOKA, LN
LEE, S

ASSESSMENT OF THE VENOUS THROMBOEMBOLISM PROTOCOL AT THE UNIVERSITY OF COLORADO HOSPITAL. LN MURAOKA, (PharmD., GS), S Lee, K Miller, K Babilonia, and T Trujillo, Aschutz Inpatient Pharmacy, University of Colorado Hospital, Denver, CO. In 2010, the University of Colorado Hospital (UCH) changed the dosing protocol for unfractionated heparin in the treatment of venous thromboembolism (VTE) from an initial 80 unit/kg bolus and 16 unit/kg/hour initial infusion, to an initial bolus of 80 units/kg and 18 units/kg/hour to better align with current international guidelines. The purpose of this study is to evaluate the performance of the revised dosing protocol in terms of achieving therapeutic anticoagulation, as well as conducting the same assessment in patients who are considered obese. Seventy-eight patients in total were included and data was abstracted and analyzed. Subsequently patients were separated into groups considered either normal weight or obese based on total body weight (< 100 kg and > 100 kg) or BMI (BMI < 25, BMI = 25-40, BMI > 40) for further analysis. Patients were excluded from the evaluation if they were on a heparin drip for less than 24 hours. The primary outcome was the time to stable therapeutic anticoagulation. Secondary outcomes included the average initial aPTT, the time to first therapeutic aPTT, and the average maintenance dose required to achieve therapeutic anticoagulation. The primary endpoint in the overall cohort was 49 + 33 hours. Results for secondary outcomes include an average initial aPTT of 122 + 78 seconds (goal aPTT for the UCH protocol is 68-90 seconds), an average time of 24.1 + 15.7 hours for the first therapeutic aPTT, and an average maintenance dose of 15.2 + 4.1 units/kg/hour to achieve stable therapeutic anticoagulation. Based on results available in this analysis, the VTE Protocol used at UCH may result in supra-therapeutic levels of anticoagulation in a majority of patients, with a subsequent effect of increasing the time to achieving stable therapeutic anticoagulation due to multiple dose adjustments. In addition, the existing protocol did not get 30% of the patients to a level of stable therapeutic anticoagulation. While some differences were noted in outcomes based on obesity status, none reached statistical significance likely due to the small sample size. Based on these results, further data collection on the protocol and adjustment to initial recommended doses is warranted.

NEIMAN, J

ROLE OF TGF β RII DELETED FIBROBLASTS IN THE TUMOR MICROENVIRONMENT. JM Neiman, (M.D. /Ph.D., SOM/GS), A Bessera, TL Cleaver, SP Malkoski, XJ Wang. Departments of Pharmacology/Pathology, University of Colorado Denver Health Science Center, Denver, Colorado. The contribution of the tumor microenvironment has increasingly been shown to play a role in tumor metastasis and progression. Genetic mutations in the tumor stroma may contribute to tumor progression, and can be utilized as a tool to better understand factors that create a tumor-promoting microenvironment. Deletion of the transforming growth factor beta receptor two (TGF β RII) is one spontaneous mutation that has been reported in stromal fibroblasts in head and neck squamous cell carcinoma (HNSCC). In the present study, we generated mouse fibroblasts with a fibroblast-specific TGF β RII deletion. We found that conditioned media from these fibroblasts caused increased invasion of HNSCC cell lines. Additionally, co-culture of TGF β RII-deleted fibroblasts under HNSCC tumor cells caused increased colony formation in soft agar assays. When we orthotopically transplanted these tumor cells into nude mice, HNSCC cells co-transplanted with TGF β RII-deleted fibroblasts showed increased metastasis to local neck lymph nodes. Histological and immunofluorescent staining of the co-transplanted tumors showed increased mitosis, proliferation, and angiogenesis. A microarray comparing wildtype and TGF β RII-deleted fibroblasts revealed candidate factors that may contribute to the above observed effects including IGF2 and FGF10, which were upregulated by 47 and 6 fold respectively. Our data suggests that TGF β RII-deleted fibroblasts secrete soluble factors that contribute to a cancer-promoting microenvironment.

NGHIEM, N

OCULAR BIOCOMPATIBILITY OF NITINOL. NT Nghiem, (M.D., MS), DA Ammar, JM Petrash, JL Olson, Department of Ophthalmology, University of Colorado, Denver, CO. Purpose: Nitinol is a nickel-titanium shape memory alloy that is widely used in implantable surgical devices. The alloy is an increasingly popular material for use in surgical instruments because of its shape-retaining characteristics, tensile strength and record of good biocompatibility. The main concern in the use of nitinol is its nickel component. Although nickel has been found to have multiple adverse effects, many studies have demonstrated good biocompatibility of nitinol in various human tissues. However, there have been no studies to assess nitinol biocompatibility in ocular tissues. The results of this study will be important in establishing the safety of a new minimally invasive nitinol suture developed for use in the eye. Methods: Cultures of human retinal pigment epithelium (ARPE-19) and human corneal endothelium (HCN E6/E7) were used. First, to establish the levels at which nickel is toxic to these ocular cells, different concentrations of nickel chloride were added to confluent cells and incubated for 3 days. Cell viability was then measured. To test for leaching effects, nitinol wire was incubated in media, which was aspirated and frozen down at different time points within an 8 week period. Both cell lines were then exposed to the different batches of media for 2 days and cell viability was measured. Cell viability for the above experiments was determined with MTT assay. In order to observe cell growth in the presence of nitinol, cells were seeded onto culture plates containing pieces of nitinol wire and allowed to grow to confluency. Results: There is a statistically significant decrease in cell viability for ARPE-19 cells at 2.5 mM nickel chloride and for HCN E6/E7 cells at 0.5 mM. The leaching experiments showed no statistically significant decrease in cell viability for either cell line. Cell growth experiments demonstrated that the cells were able to grow to confluency in the presence of and in contact with the nitinol. Conclusion: The nitinol showed minimal leaching effects and did not affect cell proliferation. Therefore, these results support the safety of ocular nitinol sutures for use in the eye. Further experimentation needs to be done to determine absolute levels of nickel that may be released from the nitinol wire.

ODA, S

LYSOPHOSPHATIDIC ACID SIGNALING INHIBITS CD8+ T CELL ACTIVATION SK Oda¹ (Ph.D., GS), Y Fujiwara², T Oravec³, G Tigyi², R Pelanda¹, and RM Torres¹. ¹Integrated Department of Immunology, University of Colorado Denver and National Jewish Health, Denver, Colorado, 80206, USA ²Department of Physiology, University of Tennessee Health Science Center Memphis, Memphis, Tennessee, 38163, USA ³Lexicon Pharmaceuticals, Woodlands, Texas, 77381, USA Lysophosphatidic acid (LPA) is a lysophospholipid that is present at low nanomolar concentrations in the plasma of healthy individuals. It has been well documented that many cancers, including ovarian, cervical, and myeloma cancers, aberrantly produce LPA resulting in elevated levels of LPA. Increased levels of LPA have been shown to be beneficial to the tumor, promoting tumorigenesis, invasion, metastases, and vascularization. However the effects of elevated levels of LPA on the adaptive immune response to the tumor have not been addressed. The immune system, and T cells in particular, are important for their ability to recognize and eliminate nascent tumors in cancer immunosurveillance and tumor rejection. However multiple inhibitory mechanisms within the tumor have been described that protect the tumor from T cells. Of note, Yervoy (ipilimumab) is an immunotherapy that was approved this year for the treatment of advanced metastatic melanoma and enhances T cell stimulation by blocking signaling to the inhibitory receptor, CTLA-4. Work in our laboratory has found that LPA inhibits CD8+ T cell activation in vitro and in vivo. Specifically, we have demonstrated that CD8+ T cells express an LPA receptor that, in the presence of LPA, is able to inhibit T cell receptor (TCR) signaling and subsequent cell activation, proliferation and function. These data document that LPA is able to negatively regulate T cell activation and function. We have preliminary data that increased LPA production by tumors functions to protect the tumor from CD8+ T cell-mediated eradication. If true, these findings would represent the first report of lysophospholipid-mediated protection of tumor from adaptive immunity and highlight a potential target for cancer treatment.

OKKOTSU, Y

PHOSPHORYLATION OF ALGR RESPONSE REGULATOR IN PSEUDOMONAS AERUGINOSA AND ITS IMPLICATIONS ON VIRULENCE. Y Okkotsu, (Ph.D., GS), FH Damron and MJ Schurr, Department of Microbiology, University of Colorado Denver Anschutz Medical Campus, Aurora, CO. The AlgR response regulator (RR), part of the AlgZ/R two-component system expressed in *Pseudomonas aeruginosa*, is a global regulator with a broad control over various virulence factors. These include, but are not limited to: alginate production, twitching motility, hydrogen cyanide production, rhamnolipid (biosurfactant) production, and biofilm formation. AlgR is phosphorylated, presumably by its putative cognate histidine kinase AlgZ, at a conserved aspartate residue at position 54 within the N-terminus receiver (REC) domain. Phosphorylation of the REC domain is believed to play a modulatory role for the C-terminus DNA-binding domain and effect transcription of its target genes. The purpose of this study was to explore the role of AlgR phosphorylation on *P. aeruginosa* virulence factor expression and overall fitness. Previous studies with other RR's (such as NtrC and OmpR from *E. coli* and *Salmonella*) have shown that a glutamate substitution of the conserved phosphoacceptor aspartate residue, structurally and functionally mimicked the phosphorylated form of the RR, while an asparagine substitution mimicked the non-phosphorylated form. In order to lock AlgR in either an active or inactive state, the algR D54E allele was introduced into PAO1 wild type lab strain and compared with WFPA8 (expressing algR D54N). Virulence factors that were previously found to be regulated by AlgR were tested with both transcriptional (reporter or qRT-PCR) and biological assays in PAO1 and its mutants, including type IV pili-mediated twitching motility and fimU promoter activity, rhII promoter activity, and rhamnolipid and pyocyanin (phenazine) production. Pathogenicity was also explored by measuring survival of Balb/c mice after inhalation. Strain PAO1 algRD54E had similar phenotypes with its parental PAO1 strain in terms of twitching motility, rhamnolipid production, pyocyanin production, and mouse virulence but had marked increases in transcription of fimU and rhII genes. Strain WFPA8 was avirulent in mice, and was defective in twitching motility, and rhamnolipid production, but had increased production of pyocyanin. The results give evidence that phosphorylation of AlgR has a functional role in *P. aeruginosa* pathogenicity.

OSBORNE, C

VIRAL GASTROENTERITIS IN CHILDREN IN COLORADO: A STOOL STORY Christina M. Osborne BA (MD, MS), Christine Robinson PhD, Stacey Schultz-Cherry PhD, Samuel R. Dominguez MD, PhD Department of Pediatrics, Infectious Diseases, University of Colorado School of Medicine Diarrhea associated with gastroenteritis is the second leading cause of death in children under age 5 outside the neonatal period and remains a significant cause of morbidity and mortality throughout the world. While bacterial and parasitic gastroenteritis have declined in prevalence due to cleaner drinking water and proper sewage disposal, the prevalence of viral gastroenteritis has not. To date, few large-scale studies on the epidemiology of viral gastroenteritis in children have been done in the United States using sensitive PCR diagnostic assays. A total of 1,182 stool samples were collected from children with gastrointestinal symptoms, which were submitted to the viral clinical virology laboratory at Children's Hospital Colorado from 2006 to 2008. Stool specimens were extracted using viral RNA kits on a BioRobot EZ1 extractor and tested for the presence of norovirus, astrovirus, adenovirus, rotavirus, sapovirus, and coronavirus using several multiplex qRT-PCR assays. All samples were pooled and analyzed in sets of six. Any positive pooled samples were tested individually for the specific virus. Influenza RNA was spiked into all samples to test for presence of PCR inhibitors. Retrospective chart review of positive samples was conducted to determine clinical and epidemiological patterns and characteristics of infection associated with each viral agent. A subset of RNA from samples negative by qRT-PCR will be pooled and submitted for next generation sequencing aimed at novel virus detection. Of pooled samples, 192/197 (97%) were positive for spiked influenza RNA; 19/197 (9%) and 97/197 (49%) of pooled samples were positive for Norovirus GI and GII respectively. Of pooled samples, 29/100 (29%) were positive for adenovirus and 29/100 (29%) were positive for rotavirus; whereas, 21/100 (21%) and 6/100 (6%) were positive for astrovirus and sapovirus respectively. Analysis of individual samples from positive pools, retrospective chart review, and sequencing are currently ongoing. To date, results indicate a significant number of stool samples are positive for RNA from various viral agents.

PARKER, J

EX VIVO EVALUATION OF TUMOR CELL MIGRATORY CHARACTERISTICS IN A HUMAN GLIOBLASTOMA SLICE CULTURE MODEL.

Department of Neurosurgery, University of Colorado, Denver, CO JJ Parker (M.D. Ph.D.,MSTP/Cancer Biology) KR Dionne, R Massarwa, M Klaassen, L Niswander, BK Kleinschmidt-DeMasters, and A Waziri. Brain invasion is a hallmark of glioblastoma (GBM) and a barrier to curative treatment. As such, models allowing for physiologically-relevant interpretation of tumor cell migratory characteristics are critical for ongoing study. We have recently developed a method for time-lapse imaging of human tumor cell migration within an ex vivo GBM organotypic slice model generated from freshly resected tumor specimens. Infection of slices with a ZsGreen-expressing retrovirus and time-lapse laser confocal imaging (10-20hr) has resulted in the identification of several distinctly-labeled populations via morphology and migratory characteristics. Stationary cells or those with highly directional migration had the predicted appearance of tumor cells, represented by long, polarized, and dynamic processes and canonical saltatory movement of the cell body. Also observed was a smaller subset of cells with the morphological features of microglia that migrated with greater speed but less directionality. Migratory paths of tumor cells were manually tracked and the resulting data were post-processed to determine mean migratory speed (total distance over time) and directionality (net path length divided by total path length). Using this system, we noted significant differences of migratory characteristics of tumor cells from different patients. Average migration speeds varied from 2.1 to 8.5 $\mu\text{m}/\text{hour}$, and greater speeds were associated with more highly directional movement (i.e. directionality ratios closer to 1.0). This study provides the first visualization of glioma cells migrating in living human brain tissue. Ongoing studies are attempting to correlate tumor cell migration data and patient-matched biomathematical metrics of invasion and proliferation obtained from MRI analysis. We predict that this system will provide new insight into the dynamics of GBM growth and dispersion. Furthermore, slice cultures generated from surgical specimens could provide a patient-specific approach to study the effects of therapeutics on the migration, proliferation and survival of glioma cells within the context of living brain tissue.

PELLEGRINO, L

INFANT BIOMARKERS OF VULNERABILITY TO SCHIZOPHRENIA: SACCADIC INTRUSIONS INTO SMOOTH PURSUIT EYE MOVEMENTS IN GENETICALLY VULNERABLE FOUR- AND SIX-MONTH-OLDS.

LD Pellegrino, (M.D.,SOM), SK Hunter, and RG Ross, Department of Psychiatry, University of Colorado, Denver, CO. Purpose: The onset of schizophrenic symptoms during adulthood is presumed to be the result of years of abnormalities in brain development, yet there are few techniques to identify the earliest steps in this aberrant developmental process. This study seeks to determine if deficits in smooth pursuit eye movement (SPEM) can identify relevant deviant brain functions as early as infancy. Deficits in SPEM have long been associated with vulnerability to schizophrenia, and this lab has previously identified them in children as young as six years of age with a family history of psychosis. Methods: Typically-developing infants with and without a family history of psychosis are tested at four and six months of age; subjects in both groups may also have experienced fetal exposure to nicotine and maternal depression in utero—two known risk factors for later development of psychotic disorders. An infrared eye-tracking system records infants' eye movements as they track a target moving across a monitor at 9 and 12.5°/second. Computerized pattern-recognition software is used to classify eye movements as SPEM or saccadic (rapid, jerky eye movements). Gain, percent time in SPEM, and saccadic subtypes are dependent measures. Results: Since SPEM deficits have been demonstrated in adults with schizophrenia, their unaffected first degree relatives, and school-age children with a parental history of psychosis, we expect that infants with a family history of psychosis will exhibit decreased gain and increased saccadic intrusions, specifically leading saccades (a subtype specific for schizophrenia). Additionally, we expect that infants exposed to nicotine or maternal depression in utero will exhibit decreased global measures of SPEM performance. Conclusion: The identification of quantifiable deficits associated with vulnerability to schizophrenia long before psychotic symptoms even begin to develop is a crucial precursor to identification, early intervention, and ultimately prevention of the disease.

PELLINEN, J

SLEEP DISRUPTION IN PRE-OBESE MICE WITH ALTERED BRAIN LIPID METABOLISM PREDISPOSES TO WEIGHT GAIN.

J Pellinen (MD, SOM), H Wang, and RH Eckel. Department of Medicine, University of Colorado, Aurora, CO. Purpose: To investigate the consequences of sleep disruption in neuron-specific lipoprotein lipase knockout (NEXLPL-KO) mice compared to that of wild type (WT) mice. Methods: 3-month-old male NEXLPL-KO and WT mice were used. Previous experiments have shown that 3-month old NEXLPL-KO mice are pre-obese, developing obesity by 4.5 mo. Four mice were recorded at a time: two NEXLPL-KO and two WT mice, age- and weight-matched, a total of six per genotype. All mice were on a 14-hour light cycle from 6:00 to 20:00. Mice were introduced to individual metabolic chambers with calorimetry, food, and water measurements recorded in 12-minute intervals. The mice acclimatized to the chambers during the first day. The second day was a baseline day, and at 14:00 on the third day the mice were removed in order to carry out the sleep deprivation experiment. Mice were sleep deprived for the last 6 hours of the light period on day 3 of the experimental protocol by the "gentle handling" method. This method involved gently touching the mice with a brush, or introducing new objects whenever behavioral signs of sleep were observed. Activity, food intake, and metabolic recordings were resumed beginning after the sleep deprivation period for the next 12 hours, after which mice were weighed and sacrificed. Results: NEXLPL-KO mice showed a greater increase in total food intake after the 6-hour sleep deprivation period (NEXLPL-KO mice increased 52.6% from baseline, compared to the WT increase of 28.8%). NEXLPL-KO mice showed a greater decrease in activity than WT mice after the 6-hour sleep deprivation period (NEXLPL-KO mice decreased 50.9% from baseline, compared to the WT decrease of 41.9%). Body weights over this short period of sleep deprivation were unchanged between groups of mice. This bidirectional change in energy balance in NEXLPL-KO mice implies a strong predisposition to weight gain. Conclusions: These data indicate that a deficiency in neuronal lipoprotein lipase signaling disrupts homeostatic responses to sleep disruption. Sleep disturbance could be a contributing environmental factor precipitating weight gain in genetically predisposed animals, and perhaps humans.

PLANK, TD

CELL TYPE SPECIFICITY AND STRUCTURAL DETERMINANTS OF IRES ACTIVITY FROM HIV-1 TRANSCRIPTS.

Terra-Dawn M. Plank, (Ph.D., GS) & Jeffrey S. Kieft, Howard Hughes Medical Institute and Department of Biochemistry and Molecular Genetics, University of Colorado, School of Medicine, Aurora, Colorado, 80045, USA Internal ribosome entry sites (IRESs) are RNA elements which recruit the translation machinery independently of the 5' end of the mRNA. The mechanism between IRES RNAs varies, and is often very intimately linked to both the global structure of the RNA and the protein factors it interacts with. Here we report our studies of IRES RNAs from HIV-1. Using the HIV-1 gag IRES as a model, we find that HIV-1 IRES activity is cell type specific, with robust activity in cell systems modeling the natural host cells for HIV-1 infection. Using both mutational analysis and structural probing techniques, we have determined that the HIV-1 gag IRES functions differently than many other viral IRESs and may require interactions with protein factors, rather than prefolded RNA structure, for function. Furthermore, using a truncation strategy, we find that IRES activity is largely conferred by a 289 nucleotide element that is present in all HIV-1 transcripts. We demonstrate for the first time that this element is functional in several additional HIV-1 transcripts including Nef, Vif, Vpr and Vpu. Using structural probing techniques, we propose a mechanism in which a common RNA element present in all HIV-1 transcripts confers the ability to initiate translation internally, and that activity from this common element is modulated by 3' nucleotides added by alternative splicing.

THE TRANSCRIPTION FACTOR ZINC FINGER PROTEIN 521 REGULATES MAST CELL

DEVELOPMENT J. Posada, (M.D., School of Medicine) K. Lukin, L. Hong, S. Warming†, J. Hagman Integrated Department of Immunology, National Jewish Health, Denver, CO 80206 and University of Colorado School of Medicine, Aurora, CO, 80045; †Genentech, Inc., South San Francisco, CA, 94080. Mast cells are primarily known for their role in immunoglobulin E (IgE)-associated immune responses in allergic reactions. However, mast cells also participate in host defense during innate and adaptive immune responses and in chronic inflammatory diseases. Although mast cells have diverse functions in immunity, little is known about transcriptional regulation and the signaling pathways that lead to their distinct roles. The transcription factor Zinc-finger protein 521 (Zfp521) plays an important role in the differentiation of hematopoietic cells including B lymphocytes and erythrocytes. Because mast cells also express Zfp521 at high levels, we sought to elucidate the role of Zfp521 in mast cell development. The Zfp521ko/ckoMcpt5-Cre mouse model was created to study the role of Zfp521 in mast cells. This model deletes coding sequences of Zfp521 genes using the Cre/loxP recombination system under the control of the mast cell protease (Mcpt)-5 promoter. The 'floxed' functional copy of Zfp521 is excised by Cre recombinase specifically in mast cells, but not in other tissues that express Zfp521. Flow cytometry was performed on cells isolated from the peritoneal cavity of Zfp521ko/ckoMcpt5-Cre mice to examine the effect of Zfp521 inactivation in the mast cell population. Zfp521ko/ckoMcpt5-Cre mice exhibited as few as one-fourth the number of peritoneal mast cells compared to littermate controls (p

EFFECTS OF ATTENTION ON TONOTOPY IN SCHIZOPHRENIA. TE Ragole (M.D., SOM), E Slason, P Teale, M Reite, and DC Rojas. Department of Psychiatry, University of Colorado School of Medicine.

A disorganization of tonotopy in the auditory cortex has been described in schizophrenia. Subjects with schizophrenia show little to no spatial organization of responses to different tone frequencies in the auditory cortex. Previous studies have shown that attending to auditory stimuli alters tonotopic distributions in healthy controls. That is, when subjects pay attention to frequency or laterality of sound, the distance between active parts of auditory cortex for each tone is dynamically increased. This study will examine the effects of attention to tone frequency and laterality on auditory cortex tonotopy in subjects with schizophrenia. The tonotopic organization for two frequencies (400Hz and 4,000Hz) of sound in 19 patients with schizophrenia and 11 comparison subjects was determined using magnetoencephalography by examining auditory evoked magnetic field dipoles. Further, the tonotopic organization for the same frequencies was determined while the subjects attended to pitch or laterality of sound. The equivalent current dipole locations were then mapped onto MRI scans of the subjects' brains. In subjects with schizophrenia, the expected result is that attention to tone frequency or laterality will have no effect on tonotopic organization in the auditory cortex. That is, the distribution of auditory evoked magnetic field dipoles will be the same as the control condition where there is no attention to stimulus. We expect to replicate the previous results of dynamic enhancement of distance between auditory evoked dipoles in the comparison subjects when attending to different frequencies or laterality. If the result is that there is no tonotopic organization of auditory cortex in patients with schizophrenia even while attending to pitch or laterality, it will suggest that the architecture underlying tonotopy in the auditory cortex is not responsive to top-down, task relevant reorganization in the same manner as in healthy subjects. This alteration in organization of the auditory cortex may in turn influence higher order cognitive processes by altering the perception of incoming auditory stimuli.

REDDI, A

MIR-9, A TUMOR SUPPRESSOR MICRORNA, TARGETS ADHERENCE JUNCTION PROTEINS RESULTING IN SKIN SQUAMOUS CELL CARCINOMA: IMPLICATIONS FOR METASTASIS AND CHEMORESISTANCE.

A Reddi (MD, MS), R White, XJ Wang Dept. of Pathology, University of Colorado, Denver, CO. Purpose of Study: Squamous cell carcinoma (SCC) is the second most common form of skin cancer. MicroRNAs (miRs), small noncoding RNAs that suppress gene expression by targeting mRNA, have emerged as potential regulators of tumor-initiating cells (TICs). In a mouse model of SCC, initiated by Kras activation and Smad4 loss, we identified miR-9 as a marker of the TIC "side population." miR-9 expression, in our model, was associated with highly metastatic passaged tumors and chemoresistance. In the present study we investigated tumor suppressor targets of miR-9 that mediate SCC oncogenesis. Methods Used: To determine SCC tumor suppressor targets of miR-9, we searched the computational algorithm TargetScan. We performed quantitative real time RT-PCR, western blotting, and immunofluorescence to validate miR targets in: primary mouse cell lines overexpressing miR-9 generated from K15.KrasG12D.Smad4^{-/-} mouse tumors and human head and neck SCC cell lines, Cal27 and FaDu. We assayed tumor growth in vivo by subcutaneous injection of tumor cell lines in the flank of immunocompetent mice. We determined chemoresistance-signaling by a transcriptional luciferase assay and a small inhibitory RNA (siRNA) screen. Summary of Results: miR-9 targets the adherence junction tumor suppressors α -catenin and E-cadherin in mouse and human head and neck SCC cell lines respectively. Injection of overexpressing miR-9 mouse tumor cells in vivo resulted in the development of highly metastatic SCC in mice. Consistently, knockdown of miR-9 in vivo resulted in muted tumor growth and inhibited metastasis. Significantly, decreased α -catenin and E-cadherin levels correlate with tumor grade and metastatic status in human SCC. Mouse cells overexpressing miR-9 had increased expression of the Abcb1a, a chemoresistance gene, and were resistant to Docetaxel induced cell death. Docetaxel increased the transcriptional response of the pro-survival signaling pathway nuclear factor-kappa B (NF κ B) in a dose-dependent manner with higher reporter activity in miR-9 overexpressing cells. siRNA inhibition of β -catenin abrogated Abcb1a expression. Conclusions: Our results suggest that miR-9 is an oncogenic microRNA in skin SCC and involved in metastasis and chemoresistance.

RITCHIE, P

IMPACT OF FETUIN ON INSULIN MEDIATED ADIPOCYTE GLUCOSE UPTAKE. PJ Ritchie, (M.D., SOM), S Mohammad, TE McGraw, Departments of Biochemistry & Surgery, Cornell Weill Medical College, Cornell University; Diabetes and Endocrinology Research Center, Columbia University Medical Center, New York, New York. Metabolic effects of bariatric surgery suggest that a circulating serum factor may be implicated in the modulation of glucose uptake in insulin sensitive tissues, and thus in insulin resistance and type 2 diabetes. Several molecular candidates have been identified that may increase or decrease insulin sensitivity, including the binding protein fetuin, a major component of serum with reported association to insulin resistance, type 2 diabetes, and regulation of insulin receptor activity. To investigate fetuin's role in the regulation of the insulin signaling pathway, we employed an in vitro GLUT4 translocation assay developed in our lab. This protein's translocation to the cell surface is one of the key actions of insulin in adipocytes and skeletal muscle, facilitating increased glucose diffusion into the cell. Our assay used quantitative immunofluorescence light microscopy to measure translocation of the GLUT4 insulin insensitive glucose transporter molecule to the cell surface under both basal and insulin stimulated conditions in mouse fibroblast differentiated adipocytes (3T3-L1 cells). Cells were electroporated with GLUT4 labeled with green fluorescent protein (GFP) and HA epitope, incubated in bovine fetuin acutely for 30 min. or overnight in media, and then incubated for 30 min. adding serial ten-fold dilutions of insulin. Cells were then fixed and stained with primary anti-HA and secondary CY3 fluorescence labeled antibodies. Fluorescence density comparison of images under two wavelengths indicated the ratio of cellular surface (CY3) to total cellular (GFP) GLUT4, averaging 30-50 cells/sample. Physiologic levels and ten-fold further serial dilutions of fetuin revealed a robust response of GLUT4 translocation to the cell surface with increasing insulin concentration, but no correlation to fetuin levels (Figure). Results and accuracy were corroborated with repetition of both 30 min. and overnight exposures. These data reveal that acute or overnight exposure to varying fetuin levels has no discernable effect on insulin-stimulated GLUT4 translocation in adipocytes, suggesting that fetuin is not the circulating serum factor relevant to insulin resistance.

SCRIMGEIOUR, L
KIMBALL, A

Context: Medicaid's home and community based services (HCBS) waiver program is expanding in Colorado as a means of providing long-term care for the elderly, blind, and disabled (EBD).

However, rural communities may face different challenges in providing these services as opposed to urban areas, particularly as the aging population expands in the future. Purpose: To gain insight into the delivery of Medicaid's HCBS-EBD waiver services in rural communities in Colorado from the perspective of providers. Methods: We conducted qualitative interviews with 11 providers in three rural regions of Colorado, each with their own single entry point (SEP) and analyzed them for central themes. Findings: The greatest challenges providers face include poor reimbursement by Medicaid and regular cuts, limited workforce with large turnover, and difficulty travelling long distances without reimbursement. However, we found that rural communities tend to provide in-kind services as needed and are often able to make do with their limited resources. Also, regarding future plans, particularly in relation to the increase in aging population, no current plans exist however they will approach challenges as they arise. Conclusion: Medicaid's HCBS-EBD waiver program in Colorado is successful in keeping many individuals out of nursing homes in rural areas, however they face challenges regarding limited resources due to lack of funding. Increased awareness of this program will help save state money spent on healthcare while improving quality of life and policy makers should keep this in mind in the upcoming decades with regard to the aging baby boomer population. Key words: Colorado, elderly, home and community based services (HCBS), Medicaid, rural

SEARLES, V

SUICIDES IN URBAN AND RURAL COUNTIES, 2006-2008 Veronica Searles, (MD, PhD),¹ Morgan Valley, MS,² Holly Hedegaard, MD, MSPH,³ Marian E. Betz, MD, MPH² ¹Medical Scientist Training Program, University of Colorado School of Medicine, Denver, CO, ² Department of Emergency Medicine, University of Colorado School of Medicine, Aurora, CO, ³ Colorado Department of Public Health and Environment, Denver, CO Suicide is the 10th leading cause of death among all ages in the United States. Suicide rates are higher in rural areas, and it has been hypothesized that increased access to firearms, lack of access to care and isolation play a role. To determine what features may influence rural suicide, we sought to: 1) Describe the demographic, socioeconomic, mental health and suicide characteristics of completed suicides in three community types characterized by level of rurality, and 2) Identify differences among victims in these three community types. Methods: We conducted a cross-sectional analysis of suicides in 16 states from 2006 to 2008, using data from the National Violent Death Reporting System. Decedent county of residence was categorized into one of three a priori groups (urban; rural adjacent to urban; and rural non-adjacent to urban). We examined associations between urban-rural setting and each suicide variable with separate logistic regressions adjusted for age, gender, race and ethnicity. Results: Of 17,504 reported suicides for which circumstances surrounding death were known, most victims were male and white, and the median age in all community types was comparable at 45-47. In all areas, most suicides were by firearm, and victims in rural non-adjacent areas were significantly more likely than urban victims to use firearms after multivariate adjustment. Victims in rural non-adjacent areas were less likely to have a psychiatric diagnosis, be in current mental health care, or have been in mental health care previously. Rural victims were also less likely to have job or financial problems as contributory factors. Conclusions: Suicide victims in rural and urban areas differed significantly. Decedents in rural areas were more likely to use a firearm as a means of suicide, less likely to have financial or job problems as contributory factors, and were less likely to have a mental health diagnosis, to be in current treatment or to have been in treatment previously. A better understanding of geographic patterns could inform suicide prevention efforts.

SIPPEL, T

ACTIVATION OF CIRCULATING NEUTROPHILS IN GLIOBLASTOMA PATIENTS. TR Sippel (PhD, GS), R Russell, M Klaassen, BK Kleinschmidt-Demasters, A Waziri. Department of Neurosurgery, University of Colorado Anschutz Medical Campus, Aurora, CO. It has become increasingly evident that inflammation plays an important role in the pathogenesis of cancer. One of the first immune cells recruited to sites of inflammation are neutrophils. In response to inflammatory stimuli, neutrophils are generally thought to undergo a defined process of adhesion to the endothelium and transmigration into the tissue. Our lab has shown that in the context of glioblastoma (GBM), this process appears to be disrupted. We have identified a population of activated neutrophils that persist within the circulation of GBM patients and act to the advantage of the tumor by inducing systemic immunosuppression. Preliminary investigation has shown that these neutrophils express elevated levels of CD66 and decreased L-selectin (1.7 and 0.2 fold respectively, compared to resting neutrophils), consistent with a state of activation. However, they express paradoxically low levels (0.3 fold compared to resting neutrophils) of CD11b, a marker involved with adherence to the endothelium that should increase with activation. This phenomenon appears to be specific to patients with GBM, as we have not observed this population in patients with other lower-grade intracranial tumors. This does not appear to be global occurrence, as resting neutrophils from GBM patients are equivalent to normal donor neutrophils in terms of baseline expression patterns of CD11b, CD66 and L-selectin, and functional analysis using formyl-Methionyl-Leucyl-Phenylalanine (fMLP)-induced activation confirmed parallel degranulation responses. Incubation of normal donor resting neutrophils with tumor conditioned media increases surface expression of CD11b and CD66 (1.6 and 1.7 fold respectively), indicating that tumor soluble factors are capable of normally activating neutrophils and do not induce the CD11b-lo phenotype observed in GBM patients. Together these data suggest that a tumor-specific interaction is responsible for this unique population of circulating, activated neutrophils in GBM patients. Further investigation into the mechanism behind the formation of this population may reveal novel, targetable aspects to prevent their development and subsequently inhibit their protumoral effect of immunosuppression.

SMUCNY, J

fMRI OF SUSTAINED ATTENTION IN SCHIZOPHRENIA. J Smucny (Ph.D., GS), L Eichman, DC Rojas, and JR Tregellas, Department of Psychiatry, University of Colorado Denver Anschutz Medical Campus, Denver, CO. Current treatments do not adequately address the cognitive symptoms of schizophrenia. One common cognitive deficit in the illness is an impairment in sustained attention. Understanding the neurobiology of this process may lead to improved treatments. To that end, we have used functional magnetic resonance imaging (fMRI) to examine neuronal response during a sustained attention to response task (SART) in the presence or absence of distracting noise. We show that in the absence of noise, the random SART recruits a network that includes the interior frontal gyrus and fusiform gyrus in both healthy controls and patients with schizophrenia. During distracting noise, however, recruitment of this network during the random SART is increased in controls, but not in patients. These results suggest that functional recruitment of cortical areas is impaired in schizophrenia during environmental noise distraction.

SNIEGOWSKI, K

The impermeability of the metazoan embryo egg shell is an important barrier in fighting the spread of parasitic infections. While an adult worm can be targeted by drugs, the egg shell protects worm embryos from most harmful substances. Following fertilization of an embryo, a trilaminar structure forms around the worm that later becomes part of the egg shell. Understanding the structure and function of the proteins involved in establishing this permeability barrier can lead to the discovery of effective anti-parasitic drugs. The scope of my project involves the characterization of five newly identified proteins hypothesized to function in establishing the structural integrity and impermeability of the *C.elegans* embryo egg shell. Preliminary data showed increased permeability to dye upon depletion of these proteins by RNAi. The European Bioinformatics Institute's InterScanPro tool was used to identify potential functional domains of each protein. Constructs containing domains of interest were cloned into 6-His tag commercial *E.coli* vectors and used to prepare 200 amino acid fragments which were subsequently purified on Nickel resin. Using immunofluorescence and light microscopy, our goal is to identify localization of the proteins during formation of the egg shell and establishment of the permeability barrier.

SORRENTINO, T

Purpose: Thirty years ago we reported our experience with abdominal vascular trauma, highlighting the critical role of hypothermia, acidosis, and coagulopathy. Damage control surgery was subsequently introduced to address this “lethal triad.” The purpose of this study is to evaluate outcomes from our most recent 6-year experience compared to 30 years ago. Methods: Patients with major abdominal vascular injuries were analyzed; the most recent 6 year period was compared with archived data from a similar 6 year period 30 years ago. Results: The number of patients with major abdominal vascular injuries decreased from 123 patients [1975-1980] to 64 patients [2004-2009]. The mean pH decreased from 7.21 to 6.86 [1975-1980 vs. 2004-2009]. In spite of increasingly protracted acidosis, mortality attributable to refractory coagulopathy has decreased from 46% to 19% [(21/46) 1975-1980 vs. (4/21) 2004-2009]. Average time from ED arrival to OR incision in patients requiring urgent laparotomy decreased from 45 to 28 minutes. There was an increase in early deaths with 42% of the mortalities from abdominal vascular injuries occurring within the first 3 hours in the 1975-1980 cohort, vs. 50% of the mortalities (18/43) occurring within the first 3 hours in the 2004-2009 cohort. Of those who died in the 3-6 hour timeframe, there was a substantial mortality shift (47% of mortalities in 1975-1980 vs. 30% in 2004-2009). Conclusion: Adoption of Damage Control has reduced mortalities for abdominal vascular injuries due to coagulopathy. However, as a result of rapid pre-hospital transport, patients continuing to die from exsanguination represent the future challenge. There was a substantial reduction of death from refractory coagulopathy that may be explained by the implementation of damage control surgery, recognizing the importance of plasma components as part of a massive transfusion protocol. Improvements in prehospital transport times reveal an increase in patients with exsanguinating injuries who died in the first 3 hours of arrival in spite of receiving an increased proportion of plasma transfusions.

	1975-1980		2004-2009	
No. of Patients with Major Abdominal Vascular Injuries	123 pts		64 pts	
Damage Control Surgery	--	--	34 pts	53%
Received >20 Units of RBC	35 pts	28%	20 pts	31%
Overall Mortality	46 pts	37%	21 pts	33%
Death Due to Exsanguination	20 pts	43%	13 pts	62%
Death Due to Coagulopathy	21 pts	46%	4 pts	19%
Average pH	7.21		6.86	
Average Temp	31.3 deg		34.1 deg	
RBC:FFF	9:1		2:3:1	

SPECHT, B

INSULIN SENSITIVITY AND CARDIOVASCULAR DISEASE RISK IN TYPE 1 DIABETES. BJ Specht, (M.D., SOM), RP Wadwa, JK Snell-Bergeon, FK Bishop, DM Maahs. Barbara Davis Center for Childhood Diabetes, University of Colorado Anschutz Medical Campus.

Cardiovascular disease (CVD) is the primary cause of death in type 1 diabetes (T1D) with many risk factors beginning in adolescence, including reduced insulin sensitivity. Our objective was to compare the relationship between insulin sensitivity (using an insulin sensitivity score [ISS]) and CVD risk factors in adolescents with and without T1D. We tested the hypotheses that CVD risk factors are increased in T1D as compared to non-DM controls and inversely associated with ISS in T1D. The ISS for adolescents ages 12-20 years with T1D (n=292; age 15.4±2.1 years; duration 8.8±3.0 years, A1c=8.9±1.6%) and non-DM controls (n=89; age 15.4±2.1 years) was determined using the model: $\log_e IS = 4.64725 - 0.02032(\text{waist, cm}) - 0.09779(\text{HbA}_{1c}, \%) - 0.00235(\text{TG, mg/dl})$. The T1D subjects were divided into ISS tertiles (ISS ≤6.83, ISS 6.83–8.59, and ISS ≥8.59) and CVD risk factors were compared between all non-DM controls and by ISS tertile in T1D subjects. Comparisons were then stratified by sex and adjusted for race-ethnicity and Tanner Stage. CVD risk factors were more atherogenic for T1D compared to non-DM adolescents (p<0.05) for body-mass index (BMI), total cholesterol, HDL-cholesterol, LDL-cholesterol (LDL-c), systolic blood pressure (SBP), diastolic blood pressure, and C-reactive protein. The ISS was significantly different between the two groups (non-DM=11.5±2.9, T1D=7.8±2.4; p<0.0001). CVD risk factors were more atherogenic in decreasing insulin sensitivity tertiles, and as compared to controls (Figure). When adjusted for race-ethnicity and Tanner Stage and stratified by sex, similar differences were seen in both males and females. T1D adolescents have decreased ISS and increased CVD risk factors compared to non-DM controls. CVD risk factors are inversely associated with ISS in T1D adolescents. Adolescence is a crucial period in the understanding and prevention of CVD risk factors in T1D and insulin sensitivity may be an important therapeutic target.

Baseline Characteristics, by ISS tertiles in T1D subjects

Variable	Non-DM N=89	T1D most IS N=99	T1D middle IS N=96	T1D least IS N=97
Age, y ^{1,2,3,4,5}	15.4±0.2	14.2±0.2	16.0±0.2	16.1±0.2
Sex, % male	46%	54%	53%	43%
Race-Ethnicity, % NHW ^a	65%	86%	82%	73%
T1D Duration, y	NA	8.8±0.3	8.9±0.3	8.6±0.3
Tanner Stage, n, % ^a				
I	4, 4%	4, 4%	3, 3%	0, 0%
II	8, 9%	19, 19%	2, 2%	1, 1%
III	12, 13%	14, 14%	7, 7%	7, 7%
IV	24, 27%	34, 34%	28, 29%	19, 20%
V	39, 44%	28, 28%	56, 58%	70, 72%
HbA1c, % ^{1,2,3,4,5,6}	5.3±0.1	7.8±0.1	8.9±0.1	10.0±0.1
BMI, kg/m ² ^{1,3,4,5,6}	22.0±0.4	20.2±0.3	22.8±0.4	25.6±0.4
BMI z-score ^{2,3,4,5,6}	0.3±0.1	0.2±0.1	0.6±0.1	1.1±0.1
Total cholesterol, mg/dl ^{3,5,6}	147±3	147±3	150±3	173±3
Triglycerides*, mg/dl ^{1,3,4,5,6}	76 (34-212)	60 (28-119)	70 (28-153)	106 (40-394)
HDL-c, mg/dl ¹	48±1	52±1	51±1	50±1
LDL-c, mg/dl ^{3,5,6}	82±3	82±2	84±3	99±3
SBP, mmHg ^{2,3,4,5,6}	109±1	109±1	114±1	117±1
DBP, mmHg ^{1,2,3,4,5,6}	64±1	66±1	68±1	71±1
CRP*, mg/dl ^{2,3,4,5,6}	0.4 (0.0-9.1)	0.3 (0.0-8.9)	0.7 (0.1-7.7)	1.3 (0.1-22.0)
ISS ^{1,2,3,4,5,6}	11.5±0.2	10.3±0.2	7.8±0.2	5.3±0.2

*geometric mean (range); NHW = non-Hispanic White; ^ap<0.05

¹non-DM vs. T1D most IS; ²non-DM vs. T1D middle IS; ³non-DM vs. T1D least IS; ⁴T1D most IS vs. T1D middle IS;

⁵T1D most IS vs. T1D least IS; ⁶T1D middle IS vs. T1D least IS, where p<0.05

STOERMER, K

GENETIC ABLATION OF MACROPHAGE ARGINASE 1 RESULTS IN ENHANCED VIRAL CLEARANCE IN A MOUSE MODEL OF ROSS RIVER VIRUS-INDUCED RHEUMATIC DISEASE. KA

Stoermer (Ph.D., GS), L Oko, AL Burrack, RG Gill, and TE Morrison, School of Medicine, University of Colorado, Denver CO. Ross River virus (RRV) and chikungunya virus (CHIKV) cause a debilitating, and often chronic, musculoskeletal inflammatory disease. To investigate the role of host immune responses in disease, we utilize mouse models of RRV and CHIKV infection in which the major pathological outcomes—arthritis, myositis, and tenosynovitis—are consistent with the clinical signs experienced by the majority of infected humans. Monocytes/macrophages constitute the major inflammatory infiltrates in the infected musculoskeletal tissues of RRV- or CHIKV-infected humans, non-human primates, and mice. Gene expression analyses of musculoskeletal tissues revealed that genes associated with immunoregulatory macrophages, including arginase 1 (Arg1), are induced by RRV and CHIKV infection. Arg1 expressing myeloid cells regulate immune, inflammatory, and tissue repair processes in malignant, fibrotic, and infectious diseases. We discovered that mice conditionally deleted for Arg1 in macrophages have significantly reduced viral loads and display less inflammation in tissues at late, but not early, times post-RRV infection, suggesting that Arg1 may inhibit viral clearance. Consistent with these studies, CD11b+F4/80+ myeloid cells sorted from muscle tissue 10 days following RRV infection express high levels of Arg1 and suppress T cell proliferation ex vivo. Two distinct pathways have been identified for inducing Arg1 in macrophages, a STAT6-dependent pathway and a STAT3-dependent pathway. In vitro, RRV infection induces IL-6, a STAT3-activating cytokine, in infected muscle cells and treatment of macrophages with infected muscle cell supernatants induces Arg1. Similarly, Arg1 induction in musculoskeletal tissues of RRV and CHIKV-infected mice correlates with the induction of the STAT3-activating cytokines IL-6 and IL-10, suggesting that arthritogenic alphaviruses activate Arg1 by a STAT3-dependent pathway. These studies have increased our understanding of the immunological mechanisms that regulate virus clearance and may aid the development of new treatments for these virus-induced diseases.

PLASMACYTOID DENDRITIC CELLS DETECT AND RESPOND TO HEPATITIS C VIRUS INFECTION

AEL Stone (Ph.D., GS), S Giugliano, L Cheng, L Golden-Mason and HR Rosen Department of Medicine, University of Colorado. Denver, CO.

Plasmacytoid dendritic cells (pDCs) represent less than 1% of circulating blood leukocytes and are key initiators of the immune response to viruses. pDCs are distinct from conventional DCs (cDCs) due to their ability to initiate interferon (IFN) production in response to viral antigens. Hepatitis C Virus (HCV) infects 170 million people and leads to a chronic infection in 80% of people infected. We currently do not understand why some patients clear the infection while the majority of infected patients develop chronicity. We hypothesized that pDCs are critical to the early response to HCV and that viral dysregulation of normal function impairs the antiviral response. Using a pDC line, we have characterized the *in vitro* response to viral antigens including RNA from the 3'UTR of HCV (pU/UC). Co-culture with viral Toll-like Receptor ligands rapidly produces Type I and III IFN mRNA, which is not mediated by IFN- α . Transfection with pU/UC, leads to rapid induction of Type I and III IFN mRNA. This increase of interferon genes is diminished by pretreatment with the HCV Core protein prior to transfection. Conditioned media from pDCs transfected with pU/UC RNA leads to a reduction in viral replication in the JFH-1/Huh7.5.1 *in vitro* HCV replication system. Together, this data suggests that pDCs may play a key role in recognition and early response to HCV infection but that this response is blocked by the viral proteins leading to viral evasion and escape.

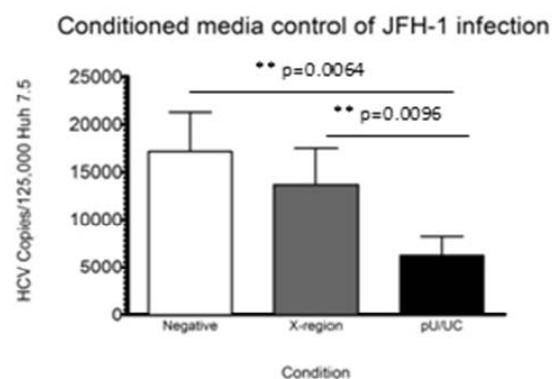


Figure 1 Conditioned media was added to Huh7.5.1 cells 24 hrs post-infection with JFH-1. Conditioned media from pDCs transfected with pU/UC lead to a reduction in viral replication. n=7

A DELPHI STUDY OF SELF COMPETENCE FOR CHILDBIRTH

T Tanner (PhD, CON), NK Lowe (PhD, CON) Purposes/Aims: The purposes of this study are to investigate expert maternity care providers' descriptions of, understanding of, and clinical experiences with women who exhibit self-competence for childbirth, and to identify the defining attributes of self-competence for childbirth. The study aims to generate knowledge for future instrument development to measure self-competence for childbirth which will enable study of factors that affect the processes and outcomes of childbirth. Method: After obtaining IRB approval, this four round Delphi study was completed with a group of 108 expert physicians, midwives, nurses, and doulas representing opinions of varying maternity care professionals possessing a wide variety of experience with childbearing women. The first round Delphi survey collected qualitative comments from panelists to five open-ended questions about their experiences with women who are self-competent for childbirth. Content analysis was undertaken revealing 550 codes which were analyzed resulting in the creation of 192 statements representing panelists' responses to these questions. These statements formed the basis for the second round survey which was comprised of these statements for ranking on a Likert scale according to how well each statement described women who are self-competent for childbirth. Data analysis revealed 49 statements reaching the level of group consensus, and identified the mean and interquartile range of each remaining statement which were then provided to panelists during the third round for reconsideration and re-ranking. Third round data analysis identified panel consensus for an additional 13 statements describing women who are self-competent for childbirth. During round four, the sixty-two total retained statements were presented to the panel for final confirmation as well as for identification of how well the statements described their nulliparous patient population. Results: At the end of the survey process, sixty-two statements were identified representing consensus about the defining attributes of self-competence for childbirth derived from this Delphi study of expert maternity care providers. Implications: Study results will provide conceptual validation and an item pool for subsequent instrument development measuring self-competence for childbirth.

UNG, T

VEGF EXPRESSION IN HUMAN MENINGIOMA IS HYPOXIA-INDUCED AND ABOLISHED BY DIGOXIN-MEDIATED HYPOXIA-INDUCIBLE FACTOR 1- α ANTAGONISM. T UNG (MD Candidate, SOM), V Tsvankin, J White, K Nag, R Russell, M Graner, A Waziri. Department of Neurosurgery, University of Colorado SOM, Aurora, CO. Meningiomas are the most prevalent benign tumor of the central nervous system. Though meningioma is often curable by surgical resection, robust tumor vascularity provides a source of potential perioperative complication as well as a potential target for therapeutic intervention. Consequently, there has been recent interest in using antagonists of vascular endothelial growth factor (VEGF) to target tumor angiogenesis. To this end, our group has conducted the first quantitative analysis of in vivo VEGF expression in meningioma, including evaluation of constitutive versus inductive VEGF expression and kinetics of in vitro hypoxia response. Using a novel extraction protocol, meningioma explants obtained from neurosurgical patients were assayed for intracellular VEGF expression; values were compared to samples obtained from low grade glioma (LGG), glioblastoma, and intracranial metastasis. In parallel, fresh meningioma explants were reduced to single-cell suspension, and primary-passage cell lines were sequentially cultured under normoxic and hypoxic conditions. Hypoxic induction of VEGF and termination of VEGF expression following return to normoxia were assayed by ELISA. In vivo VEGF levels were found to be significantly lower in meningioma than in LGG, metastasis, or glioblastoma. Furthermore, near-zero VEGF levels in short-term normoxic meningioma cultures were amplified 100-fold within sixteen hours of hypoxia induction, and remained elevated for sixteen hours after return to normoxia. Finally, hypoxic VEGF induction was abolished by concomitant treatment with the hypoxia-inducible factor 1- α (HIF1- α) antagonist digoxin. A similar pattern of hypoxia-induced elevation, as well as digoxin-induced abolition, was observed through detection HIF1- α expression by Western blotting. Results show that VEGF expression in meningioma is almost entirely hypoxia-induced, with negligible contribution from constitutive expression. These findings warrant vigilance with neoadjuvant anti-VEGF therapies, as drug withdrawal may result in induced tumor hypoxia without parallel VEGF antagonism, driving transiently high levels of VEGF and subsequent neovascularization prior to surgery.

WALKER, C

MODIFICATIONS IN DENTAL ADHESIVES BY THE ADDITION OF NANOSCALE POLYMERIC PARTICLES. CJ Walker (DS3), CS Pfeifer, JW Stansbury, School of Dental Medicine Department of Basic Science and Oral Research, University of Colorado, Denver, CO. Water absorbed into dental adhesives may cause phase-separation, decreasing mechanical properties/bond strengths. This study evaluates optical/mechanical properties of model adhesives to determine phase structure and mechanical properties as a function of water content. BisGMA and hydroxyethyl-methacrylate (60:40 weight%) were mixed with ethanol (10, 15 or 20 weight%). Saturation (SAT) and 10% undersaturation (UN) concentrations of water were determined by light transmission reduction (LTR) in an optical bench (control-CON: no water). 0.2wt% camphorquinone/0.6wt% Ethyl-4-N,N-Dimethylaminobenzoate were the initiators. LTR was followed during polymerization in bars (2x2x25mm), later tested in three-point bending. Conversion (DC) was determined by FT-IR. Data was analyzed with two-way ANOVA/Tukey's test ($\alpha=5\%$). Water required for resin saturation increased with ethanol concentration (14.5, 17.2 and 19.1wt%, for increasing ethanol content). Controls did not show appreciable LTR, except at 20% ethanol (50 \pm 15%). LTR for SAT polymerizations was: 40 \pm 1, 14 \pm 6 and 8 \pm 2% for increasing ethanol content. Same superscript within the same test indicates statistical similarity.

Ethanol	Flexural strength-MPa			Modulus-GPa			DC-%		
	CON	UN	SAT	CON	UN	SAT	CON	UN	SAT
10%	60.0 \pm 3.6 _a	29.2 \pm 3.0 _d	32.6 \pm 2.9 _c	1.7 \pm 0.2 ^a	0.7 \pm 0.0 ^d	0.9 \pm 0.0 ^c	96.4 \pm 0.3 _{ab}	95.5 \pm 0.7 _{ab}	96.6 \pm 0.5 _{ab}
15%	38.5 \pm 2.9 _b	08.1 \pm 2.0 _d	08.0 \pm 0.7 _d	1.1 \pm 0.0 ^c	0.2 \pm 0.0 ^e	1.3 \pm 0.2 ^b	97.7 \pm 0.2 _a	87.1 \pm 2.1 _c	85.5 \pm 2.8 _c
20%	33.7 \pm 2.6 _{bc}	04.8 \pm 2.1 _d	05.7 \pm 1.4 _d	1.0 \pm 0.0 ^c	0.1 \pm 0.0 ^e	0.1 \pm 0.0 ^e	97.8 \pm 0.2 _a	89.6 \pm 7.4 _{bc}	72.3 \pm 6.6 _d

The amount of water required to saturate the less hydrophilic formulations (10% ethanol) was lower. For those, phase separation during polymerization was greater, as was the decrease in mechanical properties. Next steps will include testing materials modified by the addition of hydrophobic nanogel particles. Supported by: NIH/NIDCR RC1DE020480.

WEI, PP

AUDITORY SENSORY GATING IN CHILDHOOD ONSET SCHIZOPHRENIA PATIENTS DURING REM

SLEEP. PP Wei, (M.D., SOM), S Hunter, R Ross, University of Colorado School of Medicine, Denver, CO. By early childhood, children who later develop a primary psychotic disorder such as schizophrenia already demonstrate many of the deficits that are closely associated with the disease in the adult. Children who suffer from Childhood Onset Schizophrenia (COS) also demonstrate more severe psychopathology and have worse prognoses than their adult counterparts. Psychosis-associated deficits in cognition have been shown to be related to a physiological measure of inhibitory functioning, auditory sensory gating. This endophenotype is an information processing deficit, manifesting in an abnormally increased responsiveness to repetitive stimuli, as demonstrated by a paired auditory stimulus test. Typically, the brain responds to the first stimulus but shows a significantly diminished response to the second. However, adult subjects with schizophrenia fail to show the decreased response to the second stimulus, suggesting an inability to filter repetitive stimuli due to poor cerebral inhibition. This study seeks to investigate the diminished auditory gating effect in COS patients using a P50 waveform ratio on EEG. While this experiment has successfully been done in adults, the state-dependent P50 measurement has historically been difficult to obtain in COS children due to their already heightened anxiety levels. By employing an alternative method of collecting the P50 evoked responses during rapid-eye movement (REM) sleep (previous studies have shown that P50 measurements between wakefulness and REM are comparable), such an obstacle can likely be avoided. We will recruit up to 15 diagnosed COS patients between the ages of 4-15 from whom we can record EEG measurements while paired auditory stimuli are presented through speakers near the head. Overnight recordings will be screened for REM sleep using software analysis and cross-referenced with manual inspection of characteristic eye movements and EEG data. We expect that COS patients will demonstrate defective auditory gating, similar to their adult counterparts. This goal of this study is to contribute new information to the process of COS, particularly the physiological measure of auditory gating as a reflection of cerebral inhibition capabilities, as well as to demonstrate parallels between COS and adult-onset schizophrenia.

WILSON, T

CONSUMER GENETIC SURVEYS PROVIDE NET ECONOMIC AND CLINICAL BENEFITS.

Theodore E. Wilson (MD, SOM) and Marilyn Coors Ph.D, School of Medicine, University of Colorado, Denver, CO. Over the counter genetic testing surveys have been heavily criticized by professional organizations and opinion columns. However, what is absent from current discussions about these products is a more thorough cost benefit analysis. While a one million point microarray is routinely used and is seemingly too complicated to evaluate or fully regulate in whole, if only a small minority of those SNPs had a well documented clinical use the test could be overwhelmingly beneficial. This research was a cost benefit analysis of the most likely useful SNPs included in these products and it shows that by covering for three SNPs alone (CFTR DeltaF508, Cyp282y, and Alpha1-Antitripsin Z) the small upfront economic investment in these products is warranted. The inclusion of other SNPs which may have more minor though still positive benefits is also discussed and further adds to the usefulness of these products. This poster aims to review the technology and accuracy of these products and discuss possible economic benefits provided by these tests. by these tests.