LOMA LINDA UNIVERSITY

17th Annual Basic Sciences Research Symposium

Thursday, November 13, 2014

Poster Abstracts

Poster Session A: 12:45 – 2:15 pm

Poster Session B: 2:30 – 4:00 pm

POSTER ROSTER BY LOCATION FOR STUDENTS IN SESSION A

Session	Poster Board Location	Name	Mentor
A	GS-02	Dequina Nicholas	William Langridge
A	GS-04	Leslimar Rios-Colon	Carlos Casiano
A	GS-06	H.S. Ranjan Fernando	Kevin E. Nick
A	GS-08	Leanne Woods Burnham	Carlos Casiano
A	GS-10	Christina Cajigas-Du Ross	Carlos Casiano
A	GS-12	Heather Ferguson	Nathan Wall
A	GS-14	Matthew McLain	Leonard Brand
A	GS-16	Cassia Owen	Danilyn Angeles
A	GS-18	Olivia Francis	Kimberly Payne
A	GS-20	Oliver Eshun	Williams Hayes
A	GS-22	Alexia Ximinies	Hansel Fletcher
A	GS-24	John Tan	Danilyn Angeles
A	GS-26	Brittany Hamilton	Hansel Fletcher
A	GS-28	Scott Sandy	Sean Wilson

POSTER ROSTER BY LOCATION FOR STUDENTS IN SESSION B

Session	Poster Board Location	Name	Mentor
B	GS-01	Christine Shen	Sean Wilson
B	GS-03	Leah Rowland	Eileen Brantley
B	GS-05	Yan Chen	Penelope Duerksen-Hughes
B	GS-07	Ivan Hernandez	Mary Kearns-Jonker
B	GS-09	Ryan Walden	Mary Kearns-Jonker
B	GS-11	Noemi Duran Royo	Stephen G. Dunbar
B	GS-13	John Stewart	Mary Kearns-Jonker
B	GS-15	Tania Fuentes	Mary Kearns-Jonker
B	GS-17	Abby Weldon	Kimberly Payne
B	GS-19	Ijeaoma Esiaba	Danilyn Angeles and Danilo Boskovic
B	GS-21	Andrew Crofton	Wolff Kirsch
B	GS-23	Darysbel Perez	Kylie Watts
B	GS-25	Yi Lei	Michael de Vera
B	GS-27	Ozioma Chioma	Hansel Fletcher
B	GS-29	Petreena Campbell	Eileen Brantley

POSTER ROSTER BY LOCATION FOR FACULTY / POSTDOCTORAL SCHOLARS / STAFF / OTHER

Session*	Poster Board Location	Name	Status
A-B	FS01	David A. Hessinger	Faculty
A-B	FS02	David A. Hessinger	Faculty
A-B	FS03	William Langridge	Faculty
A-B	FS04	Xiao Wen Mao	Faculty
A-B	FS05	Grant A McAuley	Faculty
A-B	FS06	Grant A McAuley	Faculty
A-B	FS07	Grant A McAuley	Faculty
A-B	FS08	Michael J. Pecaut	Faculty
A-B	FS09	Christopher Perry	Faculty
A-B	FS10	Juli Unternaehrer	Faculty
A-B	FS11	Andrew J Wroe	Faculty
A-B	FS12	Andrew J Wroe	Faculty
A-B	FS13	Andrew J Wroe	Faculty
A-B	O-01	Curtis Younger	Other
A-B	O-02	Endika Haro	Postdoc
A-B	O-03	Tino Sanchez	Postdoc
A-B	O-04	Chung-Hsiang Yuan	Postdoc
A-B	O-05	Sameer Soliman	Resident
A-B	O-06	Alexander Brunelle	Staff
A-B	O-07	Faisal Rashid1	Staff
A-B	O-08	Kenneth Williams	Staff

*All non-student poster presenters should present their poster during the last 30 minutes of Session A and the first 30 minutes of Session B. This will allow the students who are being judged to visit your posters regardless of which session they are in.

STUDENTS

#GS-1

Activation of L-type calcium channels influences calcium waves after long-term hypoxia and maturation

Christine Shen,1 Monica Romero,2 Alexander Brunelle,3 Abigail Dobyns,3 Michael Francis,4 Mark Taylor,4 Lawrence D. Longo,3 Chris G. Wilson,1,3 Sean M. Wilson 1,2,3*

Loma Linda University School of Medicine1, Advanced Imaging and Microscopy Core,2 Div. of Physiology and Pharmacology, Center for Perinatal Biology, Loma Linda CA,3 USA College of Medicine, Mobile, AL.4

L-type Ca2+ channels (CaL) mediate contraction of pulmonary arterial myocytes. FPL 64176 (FPL), a potent CaL activator, increases arterial tension beyond membrane depolarization with high K+ or direct CaL activation with Bay K 8644. Direct CaL activation with FPL, as well as modifications by long-term hypoxia and maturation, may enhance contraction of pulmonary arterial myocytes by influencing Ca2+ waves. To investigate this proposal, we manipulated CaL with FPL while recording Ca2+ waves in pulmonary arterial myocytes using confocal video microscopy of Fluo-4. Pulmonary arteries were isolated from low altitude (LA) or high altitude (HA; 3,801 m, >100 days) fetal or adult sheep. In adults, FPL decreased the area under the curve (AUC) of Ca2+ waves, number of cells firing, and interaction among ROIs in the arterial wall. The average Flou-4 fluorescence, a marker of steady-state Ca2+, was unchanged. HA decreased AUC, but increased average Ca2+ in adult myocytes. Maturation increased AUC and the number of cells with waves. FPL's decrease in Ca2+ signals suggests an uncoupling of CaL-dependent contraction from Ca2+ waves. Because the average Fluo-4 fluorescence was not correlated with changes in Ca2+ waves, the mechanisms underlying average Ca2+ and wave activity may also be distinct. These findings collectively illustrate that altitude and development cause myriad Ca2+ signaling changes that likely associate with arterial contractility and possibly other functions important to the etiology of pulmonary hypertension.

#GS-2

Identification of Anti-Palmitic Acid IgG Autoantibodies in Serum of Hispanics with Type 2 Diabetes

Dequina A. Nicholas, Lorena M. Salto, Ava M. Boston, Larry Beeson, Anthony Firek, Carlos A. Casiano, William H.R. Langridge, Zaida Cordero-MacIntyre, and Marino De Leon

Center for Health Disparities and Molecular Medicine, School of Medicine; Center for Nutrition, School of Public Health; JL Pettis VA Medical Center, Loma Linda, CA

High levels of dietary saturated fatty acids (SFAs) are detrimental to normal cellular functions and have been associated with the onset of type 2 diabetes. These SFAs can promote inflammation, secretion of the pro-inflammatory cytokine IL-1β and IgG autoantibodies. This study explored the presence of IgG autoantibodies to palmitic acid (PA) in patients with type 2 diabetes and assessed if autoantibody levels correlate with diabetes health parameters. We retrospectively analyzed serum samples from Hispanics with type 2 diabetes who participated in health education interventions that resulted in improved diabetes management. All participants tested positive for anti-PA IgG autoantibodies and showed a significant reduction following the intervention. These autoantibodies have high avidity and are specific for long chain SFAs. In addition, IL-1β

strongly correlated with dietary saturated fat in these patients. Interestingly, we found that anti-PA IgG neutralizes PA-induced secretion of IL-1β from immune cells. Thus, we show for the first time that free SFAs are recognized by IgG autoantibodies in diabetic patients. Our identification of anti-PA IgG autoantibodies in diabetes patients is strong evidence for a link between a high fat diet and FA-induced inflammation. These antibodies could potentially serve as biomarkers of disease management in diabetes patients.

#GS-3

Aryl hydrocarbon receptor ligand 5F 203 induces oxidative stress triggering DNA damage and Cytoglobin reactivation in human breast cancer cells

Leah Rowland1, Lancelot S. McLean2, Petreena Campbell1, Cheri N. Watkins1, Dain Zylstra3, Louisa H. Amis1, Lia Scott1, Crystal E. Babb1, W. Joel Livingston1, Maheswari Senthil4 and Eileen Brantley1,3

1Department of Basic Sciences, School of Medicine, 2Center for Dental Research, School of Dentistry, 3Department of Pharmaceutical and Administrative Sciences, School of Pharmacy, 4Department of Surgery, School of Medicine Loma Linda University, Loma Linda, CA 92350, USA

Despite significant advances in targeted breast cancer therapy, nearly 50,000 women succumb to breast cancer each year in the US. Thus, more effective, novel agents are needed to improve breast cancer outcomes. Emerging evidence indicate epigenetic aberrations contribute to breast tumorigenesis. Tumor suppressor gene (TSG) inactivation via epigenetic mechanisms frequently occurs during malignant transformation. Cytoglobin (CYGB) represents a putative tumor suppressor inactivated primarily due to epigenetic modifications. In particular, the CYGB promoter is hypermethylated in breast cancer cells causing inactivation and reduced CYGB expression. We have previously found that aryl hydrocarbon receptor (AhR) ligand 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203) exhibits potent cytotoxicity against breast cancer cells, promotes increases in reactive oxygen species (ROS), triggers stress-responsive kinases p38 and JNK and induces cytoglobin expression. We therefore hypothesize that 5F 203 activates ROS to trigger p38 and JNK to induce DNA damage and up-regulate cytoglobin expression to confer its anticancer actions. In this study we used quantitative real time PCR to monitor CYGB expression and Western blotting to detect protein expression. The Comet assay was used to detect DNA damage and flow cytometry was used to monitor increases in ROS. Antioxidant N-acetyl-L-cysteine (NAC) or inhibitors of p38 or JNK diminished 5F 203-mediated ROS. NAC and AhR inhibitor α-Naphthoflavone suppressed 5F 203-mediated activation of p38 and JNK depending on cell context. Cytoglobin induction in breast cancer cells paralleled their sensitivity to 5F 203. 5F 203 induced CYGB expression more so than chemotherapeutic agent Doxorubicin and epigenetic agent 5-aza-2'deoxycytidine. Inhibition of AhR, p38 or JNK pathways decreased CYGB RNA expression and DNA damage. In the future we plan to use a xenographic mouse model to study CYGB role in the tumor microenvironment. Data from our work should promote the basis for developing novel breast cancer agents that target epigenetically silenced TSGs.

#GS-4

The Stress Oncoprotein LEDGF/p75 Promotes Selective Resistance to Taxanes in DU145 Prostate Cancer Cells

Leslimar Ríos-Colón, Ivana Alicea, Anamika Basu, and Carlos A. Casiano*

Center for Health Disparities and Molecular Medicine, Loma Linda University School of Medicine, Loma Linda, CA

Prostate cancer (PCa) is the second most common type of cancer in men and the leading cause of cancer-related deaths in men over 75 years old. Furthermore, African American men have a higher incidence of PCa with more aggressive tumors and increased mortality compared to other ethnic groups. Eliminating these disparities will require innovative therapies to treat the disease and increase patient survival. Lens epithelium-derived growth factor (LEDGF/p75) is a stress survival protein that is overexpressed in PCa cells and clinical tumors, and promotes resistance to chemotherapeutic drugs used for the treatment of PCa such as Docetaxel (DTX). We have previously shown that elevated expression of LEDGF/p75 in PC3-drug resistant cells (PC3-DR) promotes selective resistance to chemotherapeutic drugs, particularly the taxanes DTX, Cabazitaxel (CTX), and Paclitaxel (PTX), but not to the classical apoptotic-inducer TRAIL. The aim of this study is to determine the effects of LEDGF/p75 overexpression or knockdown on cell viability in the presence of chemotherapeutic drugs in PCa DU145- drug resistant cells (DU145-DR). We treated DU145-sensitive and DU145-DR cell lines with increasing concentrations of taxanes and TRAIL for up to 72 hours. We also transiently knocked down the expression of LEDGF/p75 with specific small inhibitory RNA. Cell viability was assessed by MTT viability assays, clonogenic assays, and morphological examination under Hofmann Modulation Contrast microscopy. Our preliminary results suggested that knockdown of LEDGF/p75 sensitized DU145-DR cells to the chemotherapeutic drugs. In addition, we studied if increased cellular stress induced by exposure to DTX, PTX, and CTX treatment resulted in increased expression of LEDGF/p75 in a time-dependent manner. These initial results will help us to better understand the mechanisms by which LEDGF/p75 promotes chemoresistance in PCa cells, and could have implications for the development of novel therapeutic strategies for the effective treatment of advanced PCa. /

#GS-5

Chronic oxidative stress increases the integration frequency of human papillomavirus 16 in human cervical keratinocytes

Yan Chen, Maria Filippova, Valery Filippov, Vonetta Williams, Penelope Duerksen-Hughes

Division of Biochemistry, Department of Basic Sciences

Cervical cancer is the second most common cancer and the fourth most common cause of cancer death in women worldwide. Nearly all of these cases are caused by high risk HPVs (HR HPVs), of which the most prevalent type is HPV 16. Although two vaccines have been developed, those vaccines are prophylactic rather than therapeutic and cannot help patients with compromised immune systems or who already have HPV-mediated tumors. In most cervical cancers, HR HPVs are found integrated into the human genome, indicating that integration is likely to play an important role in promoting tumor development. Therefore, if we can

understand the mechanisms through which environmental factors increase the frequency of HPV integration into the host genome, we may have a unique opportunity to intercept carcinogenesis, as approaches that decrease the probability of integration could lead to fewer infections progressing to cancer. Viral integration requires DNA breaks in both the viral and the host DNA, and one mechanism that could cause this DNA damage in cells is oxidative stress. We therefore predicted that if DNA damage was increased due to high levels of ROS, the integration rate of foreign DNA or HPV should also be higher. To test this idea, we used the glutathione inhibitor L-Buthionine-sulfoximine (BSO) to increase the level of cellular oxidative stress. We also utilized two models: immortalized normal oral keratinocytes transfected with plasmid, and human cervical cell lines containing episomal HPV16. In both cases, treatment of cells with BSO increased the frequency of DNA integration. In addition, we found that BSO treatment led to an increase of ROS and DNA damage levels by inhibiting glutathione in cells. Overall, these results show that the frequency of HPV integration increases with the level of cellular oxidative stress level and the resulting DNA damage.

#GS-6

Constraints on the spatial distribution of vertebrate fossil tracks in the Navajo Sandstone, Moccasin Mt., Utah

** H. S. Ranjan Fernando, Kevin E. Nick, Gerald Bryant*

Earth and Biological Sciences, Department of Basic Sciences

Vertebrate fossil tracks are a record of animal behavior in the past. Abundant and extraordinarily preserved tracks in the Navajo Sandstone at Moccasin Mountain, Utah provide a setting to test models of: dune depositional conditions, timing, and preservation. The objective of this study is to produce a model of past depositional conditions that is constrained by information from tracks, trackways, and trampled horizons at this site. Furthermore, multiple levels of tracked surfaces in the sequence of deposition give multiple opportunities to test our models. This penecontemporaneous deformation provides a unique and significant opportunity to produce a new model that incorporates changes in the saturation state of the Navajo Sandstone. / We mapped an area of ~6000m2, between two bounding surfaces to locate dune features, tracks, trackways, and other deformation features. Track identification and interpretations rely on Ekdale, et al.,2007; Falkingham, et al.,2012; Lockley,1991, Lockley and Hunt,1995; Loope, 1986; Milner,2011; and Rainforth and Lockley,1996. Details of the preservation of deformation structures (Loope,2006; Van Loon,2009) were coded in the field by style of deformation, corresponding to different saturation states. About 200 dune foresets and more than 30 trampled horizons were found. Preliminary field work shows six taxa of track with individual track sizes ranging from 2-30cm. Trackways trend up, down, and across the slip faces and are exposed in both plan view and cross section. The spatial distribution of tracks and other deformation features are presented in a map and a series of cross sections through the study area. / Foreset orientations indicate that the dune prograded south. Preliminary assessment of the morphology of individual tracks suggests a new model that includes surface moisture conditions were critical to the formation and preservation of the tracks. Damp to wet foresets, during repetitive episodes of trampling can be explain by migration during monsoonal cycles. /

#GS-7

Effects of Short-term Hypoxia on Cardiac Progenitor Cells (CPCs)

*Ivan Hernandez*1, 3, Tania Fuentes1, Nancy Appleby1, Nahidh Hasaniya2, Leonard Bailey2 and Mary Kearns-Jonker1*

Pathology and Human Anatomy

Background: Recent advances in stem cell research involving cardiac progenitor cells (CPCs) shine light on the potential benefits of autologous stem cell transplantation for cardiac repair. Clinical trials have demonstrated that stem cell transplantation improves cardiac function in patients suffering with myocardial injuries. However, long-term, the transplanted cells fail to survive and differentiate into new tissue. Additional work is needed to identify the optimal cell types or conditions that will promote cardiovascular cell regeneration. / AKT up-regulation benefits CPC function and survival. AKT is a kinase that plays vital roles in survival, proliferation, and migration. We hypothesize that short-term hypoxia up-regulates AKT phosphorylation in CPCs and can be correlated with enhanced cell survival and motility. / Methods: CPCs were harvested from neonatal and adult human cardiac tissue using ISL1 as a marker for CPC characterization. Experimental groups were exposed to hypoxia for 6 hours at 1% O2 and 5% CO2. Cell cycle, AKT phosphorylation, and APO-BrdU analysis was performed using flow cytometry. Invasion assay was performed using Corning transwell inserts and quantified using Calcien AM. Changes in gene expression were quantified using rtPCR. / Results: CPCs exposed to short-term hypoxia showed significantly higher levels of AKT phosphorylation and genes that promote survival such as HMOX, MYC, RELA, and CCND1 (4.5 fold, p=0.00003, 12.5 fold, p=0.01, 7.6 fold, p=0.02, 9.1 fold, p=0.04, respectively) were up-regulated. Genes detrimental to the phosphorylation of AKT, such as PTEN, were down-regulated as a result of hypoxia (3.7 fold, p=0.035). CPCs exposed to hypoxia had significantly greater invasion capabilities when compared to CPCs maintained under normoxic conditions (n=10, p= 0.01). Short-term hypoxia had no significant effect on the progression of the cell cycle. / Conclusion: Prior to CPC transplantation, exposure to short-term hypoxia is feasible and may be an effective strategy to increase AKT phosphorylation, cell survival, and promote regeneration. /

#GS-8

GLUCOCORTICOIDS IN PROSTATE CANCER: ARE THEY CONTRIBUTING TO HEALTH DISPARITIES IN AFRICAN AMERICAN MEN?

Leanne Woods Burnham1, Arthur Love1, Susanne Montgomery2, Colwick Wilson2, Carlos A. Casiano1

1 Center for Health Disparities and Molecular Medicine Loma Linda University School of Medicine, Loma Linda, CA; 2 Loma Linda University School of Behavioral Health, Loma Linda, CA

The role of glucocorticoids in prostate cancer (PCa) progression has recently been explored in a few pivotal studies. Whereas androgens are traditionally known to drive PCa proliferation by activating androgen receptor, the ability of activated glucocorticoid receptor to advance this cancer to metastasis is currently under investigation. This emerging concept is valuable for two reasons. First, glucocorticoid drug therapy is presently advised for PCa patients undergoing anti-androgen treatments or chemotherapy. This recommendation may need reconsideration as emerging literature is unveiling worse prognosis correlated

with increased glucocorticoid receptor expression during androgen deprivation in patients receiving glucocorticoid drug therapy. Second, patients with aggressive PCa have higher serum levels of elevated endogenous glucocorticoid (cortisol) than early stage or localized PCa. The emerging model of glucocorticoid-driven PCa aggression is especially problematic for African American (AA) men. Men from this racial group are not only 40% more likely to have PCa and twice as likely to die from the disease, but previous studies have also discovered higher levels of serum cortisol levels in AA men with primary and metastatic PCa than in Caucasian men. In this study, we used two Caucasian prostate cell lines (PC3 and DU145) and two AA prostate cell lines (R77N and R77T) and exposed them to varying concentrations of glucocorticoids (cortisol and dexamethasone). Since stress response proteins are expressed in metastatic PCa cells, we used immunoblotting to explore a possible link between increased glucocorticoid concentration and the expression of its receptor, as well as whether glucocorticoids induce the stress response oncoprotein LEDGF/p75 which has been implicated in PCa aggressiveness. Our studies will help determine whether elevated glucocorticoids (endogenous or synthetic) induce stress oncoproteins and drive PCa aggressiveness in AA men.

#GS-9

Optimizing Seeding Conditions on Synthetic Scaffolds for Transplantation

R.C. Walden1,2, C.C. Lee3, T. Fuentes1, N. Appleby1, L. Bailey3, N. Hasaniya3, J. Hough1, and M. Kearns-Jonker1.

1Department of Pathology and Human Anatomy, Loma Linda University School of Medicine, Loma Linda, CA, 2California State University, San Bernardino, CA, and 3Molecular Matrix, Inc, Davis, CA.

Background- Stem cell therapy using cardiovascular progenitor cells (CPCs) facilitates cardiac repair in patients after a myocardial infarction. Due to limited cell retention, several types of scaffolds are being produced and tested. These scaffolds have been tested in vitro and in various animal models to determine whether administration of cardiac progenitor cells on biodegradable scaffolds improves introduction, retention, and function of the cells with the heart. / Methods- In my lab, the CPC clones were isolated from the hearts of both humans and sheep. These CPCs were KIT+, ISL1+, and SSEA4+ and can differentiate into cardiomyocytes and endothelial cells in vitro and in vivo after injection in animal models. Sheep are being used for our large animal pre-clinical studies due to the similarities in the size of human and sheep hearts. In our sheep model, our studies to date show that the ISL1+ early progenitor cells have the ability to regenerate damaged areas of the infarcted sheep heart after direct cellular injection. Biodegradable scaffolds with dimensions of 8x2mm was seeded with CPCs. Two protocols were used in the seeding of these scaffolds. The original protocol provided to me was ineffective on the first scaffold used, so a new protocol was optimized to optimize the seeding of the 2nd-4th scaffolds. After sectioning, it was determined that majority of the cells seeded in the scaffold, proliferated, and made it throughout the whole scaffold. After 2-4 weeks, the scaffolds were fixed in paraformaldehyde, embedded in paraffin, and cut into 5um sections. Sections were stained with an anti-ISL1 antibody to stain for cardiovascular progenitors, anti-Ki-67 to identify proliferative cells, anti-VWF to identify endothelial differentiation, and anti-TropT to identify progenitor cells that differentiated into cardiomyocytes. / Results- Protocol 2 showed optimal cell retention on the scaffolds. The CPCs were retained within the scaffold with evidence of differentiation into cardiomyocytes and endothelial cells after 2-4 weeks of culture on the scaffold, as demonstrated by immunohistochemistry and confocal microscopy. The cells continued to proliferate within the scaffold. / Conclusions- The close proximity of the cells within the three dimensional structure of the scaffold induced differentiation on the ISL1+ early cardiovascular progenitor cell clones into

both cardiomyocytes and endothelial cells. Cell retention was improved and further optimization is underway to minimize the time required for cellular confluency on larger scaffolds that will be used for transplantation in vivo in the sheep model of cardiovascular repair. /

#GS-10

EXPLORING THE CROSSTALK BETWEEN THE STRESS SURVIVAL ONCOPROTEIN LEDGF/p75 AND INFLAMMATORY PATHWAYS IN PROSTATE CANCER

Christina K. Cajigas-Du Ross, Anamika Basu and Carlos A. Casiano

Basic Sciences/CHDMM

Prostate cancer (PCa) is the most commonly diagnosed male cancer and the second leading cause of cancer deaths in US men. PCa is also associated with health disparities because of its disproportionately high incidence and mortality in African American men. Our group has shown Lens epithelium-derived growth factor (LEDGF/p75) expression is elevated in PCa cells and tissues. LEDGF/p75 is a stress survival transcription co-activator protecting cells against death induced by stressors such as chemotherapeutic drugs, and we have shown it promotes resistance to docetaxel (DTX)-induced non-apoptotic cell death. DTX is the first-line chemotherapeutic drug for advanced PCa, and resistance to this drug is characteristic of metastatic tumors. Recently, inflammatory cytokines such as IL6 and IL6R have been implicated in PCa aggressiveness and chemoresistance. Furthermore, overexpression of LEDGF/p75 has been shown to induce IL6 in human keratinocytes. We hypothesize LEDGF/p75 plays a role in regulating inflammatory cytokines contributing to PCa aggressiveness and chemoresistance. To identify inflammatory cytokines regulated by LEDGF/p75 in PCa cells, we monitored changes in inflammatory gene expression using real-time PCR (RT-PCR) or quantitative PCR (qPCR) inflammatory pathway specific gene arrays. LEDGF/p75 knockdown was induced in PC3 cells and in LEDGF/p75 overexpressing DTX-resistant PC3 cells (PC3-DR) using siRNAs. LEDGF/p75 knockdown was associated with down regulation of IL6 and IL6R transcripts. Western blotting analysis showed increased cellular levels of IL6 and IL6R in response to LEDGF/p75 overexpression. These studies were repeated using both DTX-sensitive and resistant DU145 PCa cells. Our results indicate that in the context of chemoresistance, LEDGF/p75 has a larger role in the regulation of inflammatory genes. To further validate these preliminary results, qPCR will be completed using in-house primers for the determined genes of interest. These studies will help elucidate mechanisms by which LEDGF/p75-induced stress and inflammatory genes contribute to chemotherapy resistance in PCa.

#GS-11

COMPARISON OF SEMI-NATURAL WITH HATCHERY NESTS AT PUNTA RATON, HONDURAS

Noemi Duran Royo and Stephen G. Dunbar

Marine Research Group. Department of Earth and Biological Sciences.

Sea turtle eggs are collected in Honduras for personal consumption and commerce, except during a protected period of 25 days, when, under enforcement by the Government, all nests are transferred to hatcheries

managed by local communities. In these hatcheries the nests are protected during the incubation period (45 days) and hatchlings are released after emergence. Punta Ratón is the main nesting beach for Olive Ridley sea turtles in Honduras, and is the location of the main hatchery with more than 200 nests per year. Previous studies have demonstrated that hatchery conditions may negatively affect hatchling development and success, as well as neonate body condition and locomotion performance. The goal of our study was to compare incubation temperatures, success, and performance, of hatchlings from nests in the hatchery at Punta Ratón with nests deposited directly on the beach by female sea turtles. We compared 4 nests from each location. Thermo-dataloggers were deployed in each nest to record temperatures during incubation. After emergence, we calculated hatching success and randomly selected 15 hatchlings from each nest to measure, weighe, and test for running speed and swimming style. We found that the incubation temperatures at the hatchery were significantly higher than at the beach, and that hatchlings from the beach were larger and performed better than those from the hatchery in swimming trials. To improve the number and quality of hatchlings released from the conservation project at Punta Raton, we recommend rather than transferring eggs to a hatchery, sections of the beach should be established and protected where turtles are moved to lay eggs and nests are kept in situ.

#GS-12

B cell lymphoma-derived exosomes are reservoirs of Inhibitors of Apoptosis proteins

Heather R. Ferguson Bennit, Malyn M. Asuncion Valenzuela, Jessica M.S. Jutzy, Nathan R. Wall

Center for Health Disparities and Molecular Medicine, Department of Biochemistry, Loma Linda University

Exosomes as a means for immunomodulation of tumor microenvironment have been the focus of recent research. These 50-150 nm sized lipid bound vesicles are secreted by many cell types, including immune cells and tumor cells and the unique protein, lipid, mRNA and miRNA contents contribute to the complex intercellular communication occurring between malignant and normal cells. Cancer patients often have increased numbers of exosomes circulating through their body, including patients with hematological malignancies, such as lymphoma. Our lab has recently discovered that exosomes from solid tumors contain Survivin, a cancer-specific member of the inhibitors of apoptosis family of proteins (IAPs). The purpose of this project is to investigate whether exosomes from hematopoietic malignancies also express Survivin, as well as other IAPs, and if the levels of these proteins and their RNA messages change under stress. Here we report IAP protein and mRNA within exosomes from lymphoma cells under basal conditions. We are currently probing exosomes from sublethally stressed cells for comparison. We hypothesize that the IAP exosomal profile may change under stressed conditions. Three human non EBV-transformed B cell lymphoma lines developed at Wayne State University were used. WSU-FSCCL (follicular small cleaved cell), DLCL-2 (diffuse large cell), and WM (Waldenstrom Macroglobulinemia) represent different levels of aggressiveness. Cells were cultured for 24 hours before exosome isolation from the conditioned media using ultracentrifugation on a sucrose cushion. Additionally, our lab has shown extracellular Survivin exerts an immune effect by inducing polarity shift to Th2 and decreasing proliferation and cytotoxicity of CD8+ T cells. These findings, as well as work of others showing exosomal contents vary when cells experience stress, form the basis of our hypothesis that DNA damage-induced stress pathways in B cell lymphomas influence the loading of exosomal cargo to increase the levels of IAPs and other immune markers. /

#GS-13

Clinically relevant small molecules inhibit anti-non-Gal IgM xenoantibody elicited in multiple pig-to-primate models of xenotransplantation

John M Stewart1, Alice F Tarantal2, Anthony JF d'Apice3,4, Peter J Cowan3,4, and Mary Kearns-Jonker1*

1Department of Human Anatomy, Loma Linda University School of Medicine, Loma Linda, CA 2Departments of Pediatrics and Cell Biology and Human Anatomy, and California National Primate Research Center, University of California, Davis, CA, USA 3Immunology Research Centre, St. Vincent's Hospital Melbourne, Victoria, Australia 4Department of Medicine, University of Melbourne, Victoria, Australia

Background: Survival of vascularized xenografts is dependent on preemptive inhibition of the anti-non-gal-α-1,3-gal (anti-non-Gal) antibody response against galactosyltransferase knockout (GTKO) porcine organs. Our analysis in multiple GTKO pig-to-primate xenotransplantation models has demonstrated that this antibody response displays limited structural diversity. That prompted our group to identify an experimental compound which selectively inhibited antibodies elicited by xenotransplantation (xenoantibodies). However, because this compound had an unknown safety profile we now extend this line of research to include small molecules with known safety profiles allowing rapid advancement to large animal models. / Methods: The NIH clinical collections of small molecules were screened in vitro by ELISA against GTKO pig endothelial cells for the ability to inhibit xenoantibody. Serum from non-immunosuppressed rhesus monkeys immunized with GTKO pig endothelial cells was utilized as a source of elicited xenoantibody for initial screening. As a proxy for selectivity, ELISAs were utilized to determine if the identified drugs inhibited natural antibodies against laminin, thyroglobulin, or ssDNA. The identified inhibitory small molecules were further tested for the ability to inhibit elicited xenoantibody from highly translational settings including non-immunosuppressed rhesus monkeys immunized with neonatal pig pancreatic islet-like cell clusters and non-immunosuppressed baboons transplanted with GTKO pig kidneys. / Results: During screening, four clinically relevant small molecules were identified to inhibit 23-64% of elicited anti-non-Gal xenoantibody. Interestingly, three of these molecules were all structurally related suggesting they have a common mechanism of inhibition. One drug displayed obvious selectivity for elicited IgM xenoantibody. Furthermore, it was able to inhibit 23-75% of elicited anti-non-Gal IgM xenoantibody from all nine animals tested and in each of four experimental settings. / Conclusions: Clinically relevant small molecule drugs with known safety profiles can inhibit xenoantibody elicited by non-Gal antigens. /

#GS-14

Solving Taphonomic Jigsaw Puzzles: Insight into the Complex Depositional History of a Lance Formation (Maastrichtian) Dinosaur Bonebed

McLain, Matthew A., Chadwick, Arthur V., Brand, Leonard, R., and Nelsen, David

Department of Earth and Biological Sciences, Loma Linda University (McLain and Brand); Southwestern Adventist University (Chadwick); Southern Adventist University (Nelsen)

A dinosaur bonebed, excavated at a site called Rose Quarry, was recently discovered in the Lance Formation (Maastrichtian) of eastern Wyoming that has bones with peculiar taphonomic signatures such as abundant

fragmentation and tooth marks. In order to understand the origins of this unusual assemblage, we studied the lithology, sedimentology, paleontology, and taphonomy of this bonebed. The disarticulated, disassociated, and commonly fragmented bones of Rose Quarry are found near the base of a trough-crossbedded sandstone unit along with mud clasts and pebbly iron-rich concretions. Some bones are very well-preserved, whereas others possess heavily damaged surfaces. These vast differences in bone preservation suggest that Rose Quarry contains a mixed assemblage. / / A model is presented to explain these taphonomic features. First, dinosaurs, turtles, and crocodilians died and their carcasses were scavenged. Scavenging dinosaurs may have trampled and crushed bones at this point, creating cracks in bones, which could have resulted in fragmentation during transport. Before weathering of the bones could occur, a flood transported additional bones to the area from a different location. This flood ripped up the bones, organic debris, and mud clasts at the scavenging location. The bones were somewhat abraded before they were finally deposited. The organic debris that was associated with the bones and mud caused numerous iron-rich concretions to form near many of the bones. This taphonomic model for Rose Quarry demonstrates the complexity that may exist for many other vertebrate bonebeds and reemphasizes the importance of careful taphonomic study in the excavation of fossils. /

#GS-15

SIMULATED MICROGRAVITY EXPOSURE IN ADULT CARDIOVASCULAR PROGENITORS

Tania Fuentes, Nancy Appleby, Ryan Walden, Leonard Bailey, Nahidh Hasaniya, and Mary Kearns-Jonker

Department of Pathology and Human Anatomy, School of Medicine

It is well known that the absence of gravity has an effect on cardiovascular function, however, the effect of zero gravity on cardiovascular progenitors is unknown. In this report we explored the hypothesis that simulated microgravity would increase aging-associated functional decline in endogenous cardiovascular progenitors. / / Adult cardiac progenitors were cultured for short (3-4 day) and long (6-9 day) periods in a 2D clinostat that simulates the absence of gravity by maintaining cells in constant rotation perpendicular to the force of gravity. After exposure to clinorotation, MAPK signaling was assessed by measuring the phosphorylation of ERK and AKT by flow cytometry. Consequences on cell function, specifically cell cycle progression, endothelial tube formation and chemotaxis were assessed by flow cytometry, tube formation assay, and transwell migration assay. Tube formation results were quantified using Wimasis software. / / Short periods of simulated microgravity significantly improved SDF-induced cell migration in adult cardiac progenitors ($p<0.05$), but did not alter cell cycle progression. There was also a significant decrease in AKT phosphorylation (4583.7 vs 3632 MFU, $P=0.01$), while levels of ERK phosphorylation remained unchanged. After exposure to long periods of microgravity, quantification of endothelial differentiation capacity using an endothelial tube formation assay revealed an increased mean loop area ($p=0.052$) and mean loop perimeter ($p=0.065$) in adult cardiac progenitors. Long periods of microgravity also decreased the level of ERK phosphorylation (9338 vs 4443 MFU $P=0.07$), but did not alter AKT phosphorylation levels. Cell cycle progression and SDF-induced cell migration were unchanged by exposure to long periods of microgravity. / / Adult cardiac progenitors differ in their biological response to short and long periods of simulated microgravity. Understanding the mechanism for the improvement of SDF-induced migration after short-term simulated microgravity may open up therapeutic options for improving adult cardiac progenitor function prior to transplantation. /

#GS-16

Fetal and intrapartum ATP degradation is increased in neonates with congenital heart disease

Esiaba I, Tan JC, Owen CE, Asmerom Y, Boskovic D, Angeles DM, Goff D

Departments of Earth and Biological Sciences, Basic Sciences and Pediatrics, School of Medicine, Loma Linda University, Loma Linda

Adenosine triphosphate (ATP) metabolism is unknown in fetus and neonates with congenital heart disease (CHD). To determine fetal and intrapartum ATP utilization in this population, we measured products of ATP degradation (hypoxanthine, xanthine, uric acid) in the cord blood of neonates with CHD (n=10) and compared it to the cord blood of normal full term neonates (n=78) using high pressure liquid chromatography. We found that neonates with CHD had significantly higher plasma uric acid concentrations (384.23 ± 28.83 vs. 267.06 ± 6.3, P = 0.003) and significantly lower plasma xanthine concentrations (2.57 ± 0.26 vs. 3.82 ± 0.39, P = 0.010) than normal neonates. Hypoxanthine concentration was not significantly different between the two groups. The conversion of hypoxanthine to xanthine and xanthine to uric acid is catalyzed by xanthine oxidase, an enzyme that is upregulated by many conditions, including inflammation and neurohormones. Further studies are required to determine the mechanism that increases ATP utilization or xanthine oxidase activity in this fragile population.

#GS-17

Surface APRIL is Elevated on Myeloid Cells and is Associated with Disease Activity in Rheumatoid Arthritis Patients

Abby Jones Weldon,1,2 Ioana Moldovan,3,4 Marven G. Cabling,3 Elvin A. Hernandez,5 Sheri Hsu,3 Jennifer Gonzalez,1 Andrea Parra,1 Abigail Benitez,1,2 Nasim Daoud,3 Keith Colburn,3 Kimberly J. Payne1,6

1Center for Health Disparities and Molecular Medicine, Loma Linda University, Loma Linda, CA, USA 2Department of Microbiology and Molecular Genetics, Loma Linda University, Loma Linda, CA, USA 3Department of Medicine, Loma Linda University, Loma Linda, CA, USA 4Division of Rheumatology, Beaver Medical Group, Redlands, CA, USA 5Department of Pharmaceutical and Administrative Sciences, School of Pharmacy, Loma Linda University, Loma Linda, CA, USA 6Department of Pathology and Human Anatomy, Loma Linda University, Loma Linda, CA, USA

Rheumatoid arthritis (RA) is a systemic autoimmune disease in which immune cells infiltrate joints and produce cytokines, ultimately leading to joint damage. Studies of inflammatory autoimmune diseases implicate the cytokine, APRIL, as a potential disease mediator. Novel surface forms of APRIL have recently been reported in human cell lines derived from myeloid malignancies. Therefore, our objective was to assess surface APRIL (CD256) expression on circulating myeloid cells in rheumatoid arthritis (RA) and to determine its relationship to patient disease activity. Peripheral blood mononuclear cells (PBMNCs) and plasma were obtained from RA patients and normal donors. PBMNCs were stained for flow cytometry to detect surface APRIL and blood cell markers to identify circulating myeloid cell subsets. Based on CD14 and CD16 phenotypes, monocyte subsets described as classical (CD14+CD16–), intermediate (CD14+CD16+), and non-

classical (CD14loCD16+) were identified. Levels of surface APRIL expression were measured by flow cytometry and median fluorescence intensity (MFI) was used for comparisons. Levels of soluble APRIL in the plasma were determined by ELISA. Disease activity was measured by Disease Activity Score out of 28 joints (DAS28). In RA patients, total myeloid cells showed expression of surface APRIL, which correlated with disease activity and with plasma APRIL levels observed in these patients. In normal donors, classical monocytes comprised >80% of circulating monocytes. However, in RA patients, the intermediate and non-classical subsets were elevated and made up the majority of circulating monocytes. In contrast to normal donors, where high levels of surface APRIL were only observed on non-classical monocytes, RA patients showed high levels of surface APRIL expression by all circulating monocyte subsets. In conclusion, surface APRIL is elevated on circulating myeloid cells in RA patients where it is highly correlated with disease activity. RA patients also showed skewing of monocytes toward subsets associated with secretion of TNF-α and/or IL-1β.

#GS-18

TSLP Regulates Expression of Genes Involved in Cell Survival in a Preclinical Xenograft Model of CRLF2 B-ALL

Olivia L. Francis, Ruijun Su, Shannalee R. Martinez, Ineavely Baez, Terry-Ann Milford, Ross Fisher, Christopher L. Morris, Xiaobing Zhang, Valeri Filippov, Sinisa Dovat, Kimberly J. Payne

Loma Linda University; Penn State Hershey

CRLF2 B-ALL is a high-risk category of the most common childhood malignancy known as Acute Lymphoblastic Leukemia (ALL) with patients experiencing a high-rate of relapse and death. These patients exhibit genetic profiles that are similar to those observed in Philadelphia chromosome+ ALL (BCR-ABL translocation+) and show overexpression of CRLF2- the receptor component for the growth factor TSLP. This defect is five times more prevalent in Hispanic children, contributing greatly to pediatric cancer health disparities. The presence of activating JAK mutations found in some patients led to the speculation that TSLP stimulation is not a factor in CRLF2 B-ALL, however, our lab and others have found that TSLP activates cell survival pathways including JAK-STAT and PI3K/AKT/mTOR pathways. Therefore, we evaluated the role of TSLP-CRLF2 interactions in vivo using a human-mouse xenograft model. Since mouse TSLP is species-specific, we engineered immune-deficient mice (NOD/SCID IL-2Rγ null) to express human TSLP and evaluated its effects on CRLF2 B-ALL cell lines and patient samples. We performed whole genome microarray using samples that were expanded in TSLP+ and TSLP- mice. Using Ingenuity Pathway Analysis, our results showed that TSLP activated cell survival pathways including the mTOR pathway and its related genes. These data were confirmed using Gene Set Enrichment Analysis. These data suggest that TSLP-induced CRLF2 signaling may contribute to leukemia cell survival in vivo. Current studies are aimed at identifying TSLP-regulated genes that can be therapeutically targeted as a part of combination therapy to successfully treat CRLF2 B-ALL and reduce the cancer health disparities for children with this disease.

#GS-19

FETAL AND INTRAPARTUM ATP DEGRADATION IS INCREASED IN NEONATES WITH CONGENITAL HEART DISEASE

Esiaba I, Tan JC, Owen CE, Asmerom Y, Gates J, Boskovic DS, Angeles DM, Goff D

Departments of Earth and Biological Sciences, Basic Sciences and Pediatrics, School of Medicine, Loma Linda University, Loma Linda

The mechanism of adenosine triphosphate (ATP) metabolism is not fully understood in fetuses and neonates with congenital heart disease (CHD). To determine fetal and intrapartum ATP utilization in this population, we measured products of ATP degradation (hypoxanthine, xanthine, uric acid) in the cord blood of neonates with CHD (n=10) and compared them to the cord blood of normal full term neonates (n=78) using high pressure liquid chromatography. We found that neonates with CHD had significantly higher plasma uric acid concentrations (384.23 ± 28.83 vs. 267.06 ± 6.3, P = 0.003) and significantly lower plasma xanthine concentrations (2.57 ± 0.26 vs. 3.82 ± 0.39, P = 0.010) than normal neonates. Hypoxanthine concentrations were not significantly different between the two groups. The conversion of hypoxanthine to xanthine and xanthine to uric acid is catalyzed by xanthine oxidase, an enzyme that is upregulated by many conditions, including inflammation and neurohormones. Further studies are required to determine the mechanism that increases ATP utilization or xanthine oxidase activity in this fragile population.

#GS-20

Probing and Landing Pressure of Mosquitoes on CO2-baited Suction Traps as a Surrogate for Host Birds at Nest or Roost Site: Implications for West Nile Virus Transmission

Oliver Eshun 1, Alec Gerry 2, and William Hayes 1*

1. Department of Earth and Biological Sciences, School of Medicine, Loma Linda University, California 2. Department of Entomology, University of Riverside, California

Factors such as mosquito attraction to specific host bird species and host birds' defensive behavior have been investigated. No study has examined how mosquito vectors are influenced by the nest or roost height of host birds in relation to distance from water and height above ground. We used nine CDC CO2 miniature traps randomly distributed at 5, 40, and 80 m from water and at 1, 3, and 6 m above ground. These were sampled twice a week for 10 nights at location 1 (adjacent to riparian habitat with trees and sheltered water) and 4 nights at location 2 (adjacent to open water) from 20 August – 4 October, 2013. We used 3 x 3 (distance x height) ANOVAS to analyze rank-transformed counts of females of five mosquito species: Aedes vexans, Anopheles franciscanus, Anopheles hermsi, and the two most abundant species which are also vectors of West Nile Virus (WNV), Culex erythrothorax and Culex tarsalis. At location 1, counts of trapped mosquitoes decreased significantly with distance from water (3 species) and height above ground (4 species); however, the effect size for height was consistently much larger than that of distance. The significant interaction for one species (A. hermsi) suggested an increase in captures with distance for the lowest (1 m) height. At location 2, all four mosquito species captured (no count for A. hermsi) showed a similar decrease in captures with height above ground, but no significant relationship with distance. The results from both locations suggest that

height of bird roost or nest site has a greater effect than distance from water in reducing probing and landing of mosquitoes thus risk for transmission of pathogens like West Nile Virus.

#GS-21

Expansion of Clinical Uses of Chitosan by Decontamination with Nitrogen Plasma

Andrew Crofton, Tyler Pender, Samuel Hudson, Angielee DiNinni, Casey Stevick, Nicholas Sanchez, and Wolff Kirsch*

Neurosurgery Center for Research and Department of Anatomy

Introduction: The goal of this study was to expand the clinical uses of chitosan (CS) – an abundant biopolymer with outstanding biomedical properties – by overcoming the key barrier preventing its internal use. This barrier is the inability of traditional sterilization techniques to decontaminate CS without negatively altering its physical and functional properties. Successful decontamination of CS with preserved functionality will enable immediate clinical testing of CS for use as a drug delivery vehicle for treating cancer and as an implantable hemostat for use in surgery. We hypothesized that non-thermal nitrogen gas plasma (NtNP), consisting of highly reactive ionized gas molecules, will sufficiently sterilize and depyrogenate CS for internal use while preserving its physical and functional properties. / Methods: Bacterial spores were treated with NtNP until a 1 x 106 reduction in spores was achieved (i.e. sterility). Before and after NtNP treatment, CS samples were assayed for (1) endotoxins using the Limulus amebocyte lysate (LAL) assay to characterize NtNP effectiveness in decontaminating CS; (2) viscosity using an Ubbelhode viscometer to identify NtNP-induced degradation of CS; and (3) bioadhesivity using an Instron tensiometer to assess preservation of CS functionality after NtNP treatment. / Results: A 1 x 106 spore reduction was achieved after 30 minutes of NtNP treatment. NtNP reduced endotoxins in CS to levels considered acceptable for implantation in humans with no reduction in viscosity or bioadhesivity. Viscosity of CS was unaltered after up to 90 minutes of NtNP exposure. / Conclusions: Our results suggest that NtNP will expand the clinical uses of CS by sufficiently decontaminating CS for implantation while preserving its physical and functional properties. Animal studies are needed to confirm that NtNP-decontaminated CS retains its in vivo functionality. Then, clinical studies testing the safety and efficacy of implanted NtNP-decontaminated CS in humans can begin. /

#GS-22

Porphyromonas gingivalis Induces PG0686, a Putative Redox Sensor, in Response to Oxidative Stress

A.D. Ximinies, Y. Dou, W. Aruni, F. Roy, L. Sandberg, H.M. Fletcher

Microbiology and Molecular Genetics

The adaptation and survival of P. gingivalis in the harsh inflammatory environment of the periodontal pocket, while inducible, may involve multiple unknown mechanisms. Transcriptome analysis in P. gingivalis demonstrated that oxidative stress modulated several classes of genes depending on the severity and duration of the exposure. There was a 4.0 upregulation of the hypothetical protein, PG0686, in P. gingivalis in the presence of 0.25 mM hydrogen peroxide for 15 minutes. Other studies have also indicated an upregulation of PG0686 in the presence of oxygen and nitric oxide. In silico analysis of PG0686 protein identified the

hemerythrin, DUF-1858 (domain of unknown function), and sensory box domains. DNA microarray analysis and Real-time RT-PCR were used to determine gene induction under oxidative stress. Flanking regions of the 1.5 kb gene were fused with the ermF antibiotic resistance cassette, and used to create a PG0686 deletion mutant, designated FLL361, by allelic exchange mutagenesis. The PG0686 gene was cloned and the histidine-tagged protein was overexpressed and purified from E. coli. Similar to the wild-type W83 strain, FLL361 was black-pigmented and showed beta hemolysis on blood agar plates. P. gingivalis FLL361 showed a 6hr generation time compared to 3hr for the wild type. Lysine-specific gingipain activity for FLL361 was reduced by 25% and 37% in both log and stationary phases, respectively, while arginine-specific gingipain activity remained unchanged when compared with the parent strain. The FLL361 mutant was more sensitive to hydrogen peroxide than W83. Our data indicate that PG0686 gene encodes for a 60 kDa protein that appears to be multimeric under native conditions and plays a role in oxidative stress resistance in P. gingivalis W83.

#GS-23

DECIPHERING KEY ELEMENTS IN THE AER2 PAS SIGNALING PATHWAY

**Darysbel Perez, Vinicius D. Cabido, Mark S. Johnson, Kylie J. Watts*

Division of Microbiology and Molecular Genetics, and The Center for Health Disparities and Molecular Medicine, Loma Linda University School of Medicine, Loma Linda, CA.

The Gram-negative bacterium Pseudomonas aeruginosa causes significant infections in compromised individuals including the lungs of cystic fibrosis patients. The ability of P. aeruginosa to survive in complex environments is aided by four chemosensory systems that sense environmental conditions and change bacterial behavior. One of these chemosensory systems, Che2, contains a single chemoreceptor named Aer2, which contains a heme-binding PAS (Per-Arnt-Sim) domain that detects diatomic gases like O2 and CO. Gas sensing by Aer2 appears to modulate P. aeruginosa's ability to establish infection. Crystal structures of the Aer2 PAS domain in ligand bound (Fe3+-CN, a proxy for O2-binding), and non-ligand bound (Fe3+) states have revealed unique residues that may stabilize gas binding (e.g., W283) or initiate conformational signaling (e.g., L264). We hypothesize that amino acid substitutions in these and other conserved PAS residues will alter ligand binding and conformational signaling. Our goal is to clarify the novel signaling mechanism used by the Aer2 PAS domain. We altered W283 and L264 by site-specific random mutagenesis, and replaced 14 conserved PAS residues with alanine. Signaling phenotypes were determined by hijacking the E. coli chemotaxis system in an otherwise chemoreceptor-less E. coli strain. Swimming behavior was observed in oxygenated and anaerobic environments and compared with the wild-type Aer2 response. Abnormal signaling behavior was observed for bacteria expressing Aer2 receptors with 14 different substitutions at W283, 12 different substitutions at L264, and alanine replacements at 12 different sites. PAS peptides with these substitutions have been purified and ligand-binding studies have commenced. Our preliminary data suggests that some W283 mutants cannot stably bind O2 but do bind CO, whereas others may have heme-binding defects. In contrast, L264 mutants can bind both O2 and CO with varying affinities. Once complete, these analyses will clarify how the heme-binding cleft within Aer2-PAS regulates ligand binding and initiates conformational signaling.

#GS-24

Comparative Effect of Repeated Doses of Oral Glucose vs. Sucrose for Procedural Pain on Urine Markers of Oxidative Stress in Preterm Neonates

Tan JBC, Esiaba I, Asmerom Y, Gates J, Hopper A, Deming D, Boskovic D, Angeles DM

Department of Basic Sciences

We previously published that a single dose of oral sucrose significantly increased plasma markers of adenosine triphosphate (ATP) utilization (xanthine) and oxidative stress (xanthine, allantoin) in neonates undergoing a clinically required heel lance. However, the effect of repeated doses of sucrose and other sweet solutions such as glucose on above markers is unknown. Using a prospective randomized double blind clinical trial, we measured urinary markers of ATP utilization and oxidative stress in preterm neonates over days of life 3-7. Subjects were randomly assigned to receive either 24% oral sucrose (n=10) or 30% oral glucose (n=6) before any tissue-damaging procedures (TDPs). TDPs occurred at an average (SD) of 9 ± 6 and study drug was administered at an average (SD) of 7 ± 6 over the study period. We found that neonates who received 24% oral sucrose had significantly higher urinary concentration of xanthine and allantoin compared to those who received 30% oral glucose ($P < 0.05$). These findings suggest that repeated administration of sucrose to relieve procedural pain might unnecessarily increase ATP utilization and markers of oxidative stress. In addition, these finding suggests that 30% oral glucose might be an alternative non-pharmacological intervention to procedural pain. Further studies are required to examine more effective ways to decrease procedural pain in preterm neonates.

#GS-25

B Cell Subset Profiling in Kidney Transplantation

Yi Lei, Abigail Benitez, Kimberly J. Payne, Michael De Vera

Transplantation Institute and Department of Anatomy, SM

Kidney transplantation is a life-saving treatment for end-stage renal disease. Maintenance of long-term host tolerance to a transplanted kidney remains a problem as outcome varies from patient to patient. Hence, early prediction of transplant kidney rejection can provide a basis for modulating treatment to prevent rejection. One type of kidney rejection is mediated by the production of host antibodies against the transplant donor organ known as antibody mediated rejection (AMR). Early detection of AMR is critical to prevent rejection. Furthermore, recent reports in lupus patients indicate that B cell subset populations can be used as biomarkers for immune dysregulation. B cell subsets display unique effector functions as well as production of specific types of antibodies. An abnormal increase or decrease of a specific subset within the B cell pool becomes an indicator or “window” into the immune status of the individual patient. Our overarching hypothesis is that differences in B cell subsets will distinguish kidney transplant patients who 1) have good versus poor graft function during the first 6 months post-transplant, 2) are at risk of developing AMR. Our study will be novel in the transplant field as extensive B cell subset characterization has never been done in transplant patients. One outcome of this study will be the development of a model in order to assess specific memory B cell populations that can be used in transplantation and rheumatology studies. The ultimate goal of

this study is to utilize the basic science principles of B cell population dynamics and correlate these findings with patient clinical outcomes. In turn, these translational studies will provide foundations for improving transplant diagnosis and patient-specific therapy as well as clinically-based questions for basic science research.

#GS-26

The Role of the Filifactor alocis RTX Gene Cluster in the Secretion of Immune-Modulating Proteins

Brittany Hamilton, Ozioma Chioma, Yeutan Dou, Wilson Aruni and Hansel M. Fletcher

School of Medicine, Basic Sciences, Division of Microbiology and Molecular Genetics

Filifactor alocis is a Gram-positive organism identified as a marker organism for periodontal disease, a condition that involves the inflammation of the periodontal pocket. Co-infection of F. alocis with Porphyromonas gingivalis, an important organism implicated in periodontitis, showed an up-regulation of proteins that may be linked to the virulence of F. alocis. These proteins include the Type I Secretion System (T1SS) proteins, which secrete immune-modulating repeat-in-toxin (RTX) proteins and are normally only identified in Gram-negative bacteria. RTX cluster proteins, specifically the leukotoxin translocation ATP-binding protein [LktB-HMPREF0389_01580], the Hypothetical protein [RtxA-related protein-HMPREF0389_01695], and the Hypothetical protein [TolC ortholog-HMPREF0389_01547], showed a higher expression following co-infection with P. gingivalis. These results lead to the hypothesis that the subunits comprising the T1SS are functional in F. alocis and could play a role in the secretion of immune-modulating proteins. In order to test the functionality of these proteins, we have begun a study of gene expression levels of the T1SS proteins during F. alocis mono-infection and co-infection with P. ginigivalis. We have also started to generate isogenic mutants of the genes coding for T1SS subunits using overlapping extension PCR. The mutants will be characterized for their functions including secretion of immune modulatory proteins. This study will expand the knowledge of unique F. alocis related secretion systems and highlight their role in virulence and host immune modulation.

#GS-27

F. alocis mediates pathogen interaction and virulence through adherence and invasion by collagen related proteins.

Ozioma Chioma, A. Wilson Aruni, Yuetan Dou, Hansel M. Fletcher

Division of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, California 92354, USA

Adhesion of bacteria to the components of the host's extracellular matrix (ECM) is the initial step to virulence. Bacteria target host proteins such as collagen, fibrinogen and fibronectin. This is facilitated by microbial surface component-recognizing adhesion matrix molecules (MSCRAMMs). F. alocis possesses cell wall anchor proteins that may recognize/bind collagen, and expression of these proteins are up-regulated during co-infection with epithelial cells. There is a gap in our understanding of the role of F. alocis collagen related

MSCRAMMs in host tissue colonization during periodontal disease. We hypothesize that F.alocis MSCRAMMs play a role in tissue colonization by binding/degrading host collagen, thereby gaining access into the host. In silico analysis and protein modeling of collagen associated MSCRAMMs of F. alocis - collagen adhesin protein (HMPREF0389_01006) and Protease (HMPREF0389_00122), showed collagen binding and collagenase motifs respectively, as well as molecular relatedness to MSCRAMMs of other pathogenic bacteria. Isogenic mutants were created by overlapping extension PCR using the tetQ cassette. The isogenic mutants of the protease (HMPREF0389_00122) and collagen adhesin protein (HMPREF0389_01006) were designated FLL211 and FLL212 respectively. FLL212 showed white colonies, which displayed beta-hemolysis, in contrast to small translucent, non-hemolytic wild type colonies. In addition to reduced growth in both mutants under normal conditions, there was no variation in growth during oxidative stress compared to the wild type. An increase in adherence, and biofilm formation was noted in FLL212; but, invasion studies showed a decrease in the ability of both mutants to invade the epithelial cells. A comparative proteome analysis showed nine proteins that were missing from the membrane of FLL212 compared to the wild type strain; however the results need further confirmation. Taken together, our observations suggest that collagen related MSCRAMMs of F. alocis could modulate other virulence factors and play a vital role in virulence and pathogenesis of F. alocis. /

#GS-28

Acute hypoxia-induced endothelium-dependent suppression of Ca2+ waves in ovine pulmonary artery smooth muscle cells

Scott Sandy, Monica Romero, Ricardo Paez, Michael Francis, Mark S. Taylor, Lawrence D. Longo, Sean M. Wilson*

California Baptist University (Sandy); Advanced Imaging and Microscopy Facility, Loma Linda University School of Medicine (Romero); Center for Health Disparities and Molecular Medicine, Loma Linda University School of Medicine (Paez); Department of Physiology, University of South Alabama College of Medicine (Francis, Taylor); Center for Perinatal Biology, Loma Linda University School of Medicine (Longo, Wilson)

Ca2+ is an important ion in cellular signaling, especially within the pulmonary artery smooth muscle cells (PASMC). Calcium ions induce smooth muscle contraction of the pulmonary arteries during hypoxia. A sustained rise and fall in intracellular [Ca2+] is called a Ca2+ wave, and is a common signaling mechanism within the PASMC. To better understand these Ca2+ signals under hypoxia, and the role of functional endothelium in producing them, we performed confocal imaging using Fluo-4 calcium indicator dye loaded into adult sheep PASMC. The present studies tested the hypothesis that functional endothelium modifies Ca+ waves during acute hypoxia (~4% O2 for 30 minutes), as little is known about how the endothelium affects Ca2+ signaling in PASMC. Automated analyses were used to examine endothelium-intact and endothelium-denuded arteries in order to determine the magnitude and duration of the Ca2+ waves. Under normoxic conditions, endothelium removal decreased the amplitude and area under the curve (AUC) of Ca2+ waves, but did not significantly impact the duration of those normoxic Ca2+ waves. Acute hypoxia in endothelium-intact arteries suppressed the amplitude, AUC, and duration of Ca2+ waves compared to the normoxic control. With the exception of a slightly reduced amplitude, endothelium removal prevented the decreases in Ca2+ wave magnitude and duration under acute hypoxia. These data illustrate that functional endothelium facilitates acute hypoxia-induced suppression of Ca2+ waves, an effect that could influence either smooth muscle contraction or gene transcription, known respectively as excitation-contraction coupling or excitation-transcription coupling.

#GS-29

Aminoflavone promotes breast tumor differentiation and inhibits breast cancer stem cell growth in vivo

Petreena Campbell1, Eileen Brantley1,3, Mariana A. Callero2, Damien E. Berardi2, Dain Zylstra3, Laura Todaro2, Louisa Amis1, Marina Simian2, Andrea Loaiza-Perez2 and Ubaldo Soto1

1Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA, US 2Research Area, Institute of Oncology, ''Angel H. Roffo'', University of Buenos Aires, Ciudad de Buenos Aires, Argentina 3Department of Pharmaceutical and Administrative Sciences, Loma Linda University School of Pharmacy, Loma Linda, CA, US

Despite significant advances in breast cancer treatment, the American Cancer Society reveals breast cancer-related deaths among American women has remained largely unchanged over the past decade. These deaths have been largely attributed to relapse and resistance to traditional therapies. Cells within breast tumors known as breast cancer stem cells (BCSCs) have been identified as the underlying factor associated with tumor recurrence and therapy resistance. Hence, there is need for novel agents which target BCSCs. Our lab and others have demonstrated that the flavonoid, Aminoflavone (AF), exhibits potent anticancer activity and disrupts the three-dimensional cluster of BCSCs (mammospheres) in vitro. Consequently, we hypothesize that AF blocks the growth of bulk tumors, BCSCs and their capacity to self-renew in vivo. To investigate the in vivo efficacy of AF, we used a spontaneous MO5 model developed by Dr. Marina Simian and colleagues (Buenos Aires, Argentina). MO5 breast cancer cells were injected into female mice which were later exposed to AF through intraperitoneal injections. FACS analysis and immunohistochemistry were used to identify BCSCs in extracted tumor cell suspensions and to carry out histological analysis of the tumors respectively. After AF treatment, tumor size and growth rate decreased compared to control animals. The number of cells that stained positive for BCSCs was significantly lower in animals treated with AF. Tumors obtained from animals exposed to AF also demonstrated diminished capacity to form mammospheres in vitro and contained more differentiated cells. These data suggest AF thwarts bulk tumor growth and diminishes the quantity and self-renewal capacity of BCSCs in vivo. These findings show that AF kills and differentiates BCSCs revealing a novel mechanism by which this investigational agent confers its anticancer actions. AF thus exhibits the potential to counteract resistance and prevent relapse associated with traditional cancer therapies.

FACULTY

#F-01

Channel differences in ovine near-term fetus and adult small pulmonary arteries

David A. Hessinger, Glyne U. Thorington, Irina Sokolova, Lawrence D. Longo

Division of Physiology, Department of Basic Sciences and Center for Perinatal Biology, School of Medicine, Loma Linda University

Pulmonary vasodilatation at birth underlies fetal transition to lung breathing. Failure to vasodilate causes persistent pulmonary hypertension of the newborn, persistent patency of the ductus arteriosus and foramen ovale, and bronchopulmonary dysplasia-related hypoxia, often with dire consequences. The mechanism of

pulmonary vasodilatation at birth is poorly understood. Pulmonary artery smooth muscle cells (PASMCs) isolated from 4th and 5th order resistance vessels of ovine near-term fetus exhibited resting membrane potentials 8 mV more depolarized than adult ($P<0.05$). Although whole-cell Kv current densities for voltage-clamped fetal and adult PASMCs were similar, large-conductance, calcium-activated K+ (BK) current densities for fetal cells were significantly lower than adult at moderate potentials (i.e. +20 to +60 mV), but equaled those of adult at higher potentials. Fetal PASMCs expressed twice the pore-forming BKα subunit protein as adult, but only half as much accessary BKβ subunit on Western immunoblots. Cell surface β-l expression was also significantly lower in fetal PASMCs by flow cytometry. Since optimal BK channel activity occurs at 1:1 β-to-α stoichiometry, our results suggested the near-term fetus expressed BK β:α ratios about ¼ that of adult. In contrast to measured current densities and protein levels, both BKα and BKβ RNA transcripts were two-fold higher in near-term fetus than adult by qRT-PCR. These findings suggest that near-term fetal PASMCs are more depolarized and exhibit lower BK activity due to lower BKβ subunit expression, but may be poised to express higher levels of BKβ to enhance BK activity and promote pulmonary vasodilatation at birth.

#F-02

Role of Kv1.3 channels in sea anemone model of satiety

Glyne U. Thorington, Virginia McAuley, and David A. Hessinger

Division of Physiology, Department of Basic Sciences, SM, LLU

Obesity is a major risk factor for the leading causes of death and recent findings indicate appetite control mechanisms are the primary cause; not lack of exercise. Competing hunger and satiety pathways control appetite. While little is known about either, less is known about satiety. Mouse knockout studies implicate Kv1.3 channels in mammalian satiety, but the complexity of the mammalian brain makes studying the role of Kv1.3 channels difficult. We, therefore, studied the role of Kv1.3 channels in a sea anemone model of satiety. The anemone is a suitable model organism because, in addition to being simpler, it displays a well-characterized and robust satiety response and uses cyclic AMP-based pathways and Kv1.3 channels, both of which are shared with mammals. Additionally, anemones secrete selective Kv1.3 channel blockers as nematocysts components to capture food prey. Our overall hypothesis is that sea anemone nematocyst venom contains a non-toxic satiety hormone that stimulates conserved satiation pathways in anemones and mammals by inhibiting Kv1.3 channels. We partially test our hypothesis by comparing the satiety effects of 18 selective Kv channel blockers in an anemone (Haliplanella luciae) satiety assay. Three of the Kv channel blockers activate satiety: two are anemone Kv1.3 channel blockers; the other is a non-selective Kv blocker, 4-aminopyridine (4-AP). We implicate the mechanism of action of 4-AP in isolated sensory cells from H. luciae tentacles using conventional whole-cell patch clamp. One such anemone Kv1.3 channel blocker and 4-AP have recently been shown to inhibit weight gain in mice and rats, respectively. Together these and our findings open the door to developing a novel class of satiety agents to treat clinical obesity. In the future, the H. luciae satiety assay may serve as a high-throughput bioassay for screening potential satiety agonists and elucidating conserved satiety signaling pathways

#F-03

Induction of Indoleamine 2, 3 Dioxygenase in Human Dendritic Cells by a Cholera Toxin B Subunit - Proinsulin Vaccine

Jacques C. Mbongue1, 2, Dequina Nicholas1,3, Kangling Zhang3,4, Brittany N. Hamilton1,5, Marco Larios1, Guangyu Zhang3, Kazuo Umezawa6, Anthony Firek7, and William H.R. Langridge1,3*

1 Center for Health Disparities and Molecular Medicine, Loma Linda University School of Medicine, Loma Linda, CA 92354, USA 2 Loma Linda University School of Medicine, Department of Basic Sciences, Division of Physiology, Loma Linda, CA 92354, USA 3 Loma Linda University School of Medicine, Department of Basic Sciences, Division of Biochemistry, Loma Linda, CA 92354, USA 4 Department of Pharmacology and Toxicology, School of Medicine, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555 USA 5 Loma Linda University School of Medicine, Department of Basic Sciences, Division of Microbiology and Molecular Genetics, Loma Linda, CA 92354, USA 6 Aichi Medical University, School of Medicine, Department of Molecular Target Medicine Screening, Nagakute, Aichi 480-1195, Japan. 7 Endocrinology Section, JL Pettis Memorial VA Medical Center, Loma Linda, CA 92354, USA

Abstract / Dendritic cells (DC) interact with naïve T cells to regulate the delicate balance between immunity and tolerance required to maintain immunological homeostasis. To study mechanisms underlying chimeric vaccine suppression of tissue specific autoimmunity, immature human dendritic cells (iDC) were inoculated with a chimeric fusion protein vaccine containing the pancreatic β-cell auto-antigen proinsulin linked to a mucosal adjuvant the cholera toxin B subunit (CTB-INS). Proteomic analysis of vaccine inoculated DCs revealed strong up-regulation of the tryptophan catabolic enzyme indoleamine 2, 3 dioxygenase (IDO). Increased biosynthesis of the immunosuppressive enzyme was detected in DCs inoculated with the CTB-INS fusion protein but not in DCs inoculated with proinsulin, CTB, or an unlinked combination of the two proteins. Immunoblot and PCR analyses of vaccine treated DCs detected IDO mRNA by 3 hours and IDO protein synthesis by 6 hours after vaccine inoculation. Determination of IDO activity by measurement of tryptophan degradation products (kynurenines) showed increased tryptophan cleavage into N-formyl kynurenine in vaccinated DCs. Inoculation with the fusion protein did not interfere with monocyte differentiation into DCs, suggesting the vaccine can function safely in the human immune system. Treatment of vaccinated DCs with the N#F-κB inhibitors ACHP or DHMEQ inhibited IDO biosynthesis, suggesting a role for N#F-κB signaling in vaccine up-regulation of dendritic cell IDO. Heat map analysis of the proteomic data revealed an overall down-regulation of vaccinated DC functions, suggesting vaccine suppression of DC maturation. Together, our experimental data indicate that CTB-INS vaccine induction of IDO biosynthesis in human DCs results in the inhibition of DC maturation generating a durable state of immunological tolerance that can safely and effectively protect prediabetic patients against development of type 1 diabetes autoimmunity. /

#F-04

Detection of Early-stage Apoptosis in Mouse Brain after Combined Exposure to Simulated Microgravity and Radiation

Xiao Wen Mao, Michael J. Pecaut, Mary Campbell-Beachler, Peter Gifford and Daila S. Gridley

Dept of Basic Sciences, LLU

Prolonged exposure to actual or simulated microgravity and low-dose radiation are known to produce a number of various physiological adaptations and neurological disturbances in the central nervous system (CNS). However, our knowledge about these disturbances following combined microgravity and low-dose rate (LDR) radiation exposure is very limited. The purpose of the present study is to examine the induction of apoptosis-associated protein profiles in the brain after combined exposure to simulated microgravity and low-dose radiation. LDR γ-irradiation using 57Co plates (0.04Gy at 0.01cGy/h) was delivered to the whole-body of mature 6 month old adult C57BL/6 mice (n=4-6/group) to simulate the radiation component. Anti-orthostatic tail suspension was used to model the unloading, fluid shift, and physiological stress aspects of the microgravity component. Mice were hindlimb suspended and/or irradiated for 21 days. Brains were isolated for characterization of apoptosis-associated proteins 7 days after the completion of irradiation and unloading. Brain cell lysates from each group were incubated overnight with RayBio Human Apoptosis Antibody Array Membranes. Biotinylated antibodies were used to detect bound proteins and signals were visualized by chemiluminescence. BAD, FAS and FASL proteins were significantly activated ($p<0.05$) at the 7-day time point in the unloaded group compared to controls. There were statistical trends for differences between groups noted in BAX, BCL-2 and HSP27 ($P<0.1$), where hindlimb unloaded mice had a greater than 2-3 fold higher expression level compared with radiation alone or unloaded + radiation groups. These proteins play important roles in induction of apoptosis. This study demonstrates that hindlimb unloading may induce early apoptosis in mouse brain and radiation may exacerbate simulated microgravity-induced damage in brain structure and tissue function. Combined exposure to simulated microgravity and low-dose radiation-induced apoptosis will be examined for longer time points in our future studies. This study is supported by NASA grant NNX13AL97G.

#F-05

Magnetically Focused Proton Irradiation of Small Volume Targets

Grant A McAuley, James M Slater, Andrew J Wroe

LLU, LLU, LLUMC

Background: Advances in imaging technologies are fueling a trend in radiation medicine necessitating the irradiation of smaller targets with greater conformity. However, as field size decreases, the peak to entrance dose performance and penumbra of proton beams is degraded by beam broadening due to multiple Coulomb scattering (MCS). Magnetic focusing of protons immediately before entrance into tissue could be used to counteract MCS leading to improved therapeutic ratios and decreased treatment times.

Methods: Magnets consisting of 24 segments of radiation hard samarium-cobalt adhered into hollow cylinders were manufactured. Two focusing magnets were placed on a positioning track on our Gantry 1 treatment table. Proton beams with energies of 127 and 157 MeV, 0, 15, and 30 mm modulation, and 5 and 8 mm initial diameters were delivered to a water tank using single-stage scattering. Depth dose distributions were measured using a PTW PR60020 diode detector and transverse profiles were measured with Gafchromic EBT2 film. Monte Carlo simulations were also performed - both for comparison with experimental data and to further explore the potential of focusing in the context of proton radiosurgery.

Results: Results and analyses of experimental data and comparisons with analogous Monte Carlo simulations are ongoing. Preliminary Monte Carlo simulations of 5 mm diameter 100 MeV beams focused with 400 T/m magnet gradients produced beam-spots at the Bragg peak that were comparable to unfocused 2 mm collimated beams. The focused beams display an ~9x more efficient dose to target delivery, 21% larger peak

to entrance ratios, 11% reduced Bragg depth major axis penumbras, 16% reduced Bragg depth minor axis penumbras, and 36% reduced entrance minor axis penumbras, compared to the 2 mm collimated beams.

Conclusion: The efficient production of small target beam spots with reduced entrance dose and penumbra has important clinical implications and our results suggest that rare earth focusing magnet assemblies could reduce skin dose and beam number while delivering dose to millimeter-sized nominally spherical radiosurgery targets over a shorter time compared to unfocused beams. Immediate clinical applications include those associated with proton radiosurgery and functional radiosurgery of the brain and spine, however expanded treatment sites can be envisaged as imaging technologies continue to improve.

#F-06

Evaluation of the dosimetric properties of a diode detector to proton radiosurgery

Grant A McAuley, Anthony V Teran, Jerry D Slater, James M Slater, Andrew J Wroe

LLU, San Diego State University, LLUMC, LLU, LLUMC

Background: Small fields typically encountered in proton radiosurgery require high spatial resolution dosimetric measurements, especially below 1-2 cm diameters where the limits of standard metrology devices are exceeded. Radiochromic film provides high resolution but exhibits a LET dependent response and requires post processing and special handling. Promising alternatives are diode detectors with small sensitive volumes (SV) that are capable of high resolution and real time dose acquisition. In this study we evaluated the PTW PR60020 proton dosimetry diode in radiation fields relevant to radiosurgery applications.

Methods: Beams with energies of 127 and 157 MeV and initial diameters of 8 and 20 mm were delivered using single-stage scattering and three modulations (0, 15, and 30 mm) to a water tank in our Gantry 1 treatment room. Precursory experiments evaluated diode response as a function of dose, dose rate, and diode orientation (ie, diode positioned with beam parallel to (axial mode), or perpendicular to detector axis (edge-on mode)). Depth dose profiles were measured using the diode in axial mode or a PTW Markus N23343 ion chamber. Transverse dose profiles were measured using the diode in edge-on mode or EBT2 film. Finally, analogous Monte Carlo simulations were performed using Geant4.

Results: Diode response was linear with respect to dose, uniform with dose rate, and essentially independent of orientation. Close agreement of ion chamber ('gold standard') and diode depth dose profiles suggests diode LET dependence was negligible. Similarly, plots of the diode to Markus dose ratio vs dose-weighted lineal energy suggest that diode response is essentially LET independent downstream and over the SOBP of the Markus depth dose curve. Also, while not possible with the ion chamber, accurate diode dose measurements of 8 mm diameter beams were obtained. Finally, in edge-on mode data was acquired with spacing as low as 0.25 mm in the penumbra of transverse profiles.

Conclusion: The PR60020 diode response was linear with dose and dose rate, and not dependent on orientation. Comparison with 'gold standard' ion chamber data suggests a negligible LET dependence over particle ranges relevant for clinical radiosurgery. The small SV allows measurements of small fields without partial volume effects. Edge-on, the diode is capable of sub-millimeter resolution (on par with film) that is essential for small fields and high dose gradients (eg, penumbra, distal edge).

#F-07

Single-plane Magnetically Focused Narrow Elongated Small Field Proton Beams

Grant A McAuley, James M Slater, Andrew J Wroe

LLU, LLU, LLUMC

Background: The original clinical goal of the project was to deliver narrow elongated proton beams to the spinal cord for potential neuropathic pain treatments. Previous Monte Carlo simulations suggested that using a single quadrapole magnet could produce beams of elliptical cross section with superior dose distribution properties and greater efficiencies than collimated beams (McAuley et al Phys. Med. Biol. 58 (2013)). The parameters of the simulated magnets were chosen to mimic k=3 Halbach cylinders that are available commercially as assemblies of rare earth permanent magnetic materials. In the present study, results from experiments with actual prototype magnets are presented.

Methods: Magnets consisting of 24 segments of radiation hard samarium-cobalt (Sm2Co17) adhered into hollow cylinders were manufactured. A single focusing magnet was placed on a positioning track on our Gantry 1 treatment table and 15, 12 and 8 mm diameter proton beams with energies and modulation relevant to clinical radiosurgery (127 to 186 MeV, and 0 to 30 mm modulation) were delivered to a water tank. Dose distributions were measured using a PTW diode detector and Gafchromic EBT2 film. Longitudinal and transverse dose profiles were analyzed and compared to data from Monte Carlo simulations analogous to experiments.

Results: The narrow elongated focused beam spots showed high elliptical symmetry indicating high magnet quality. Monte Carlo simulations were in good agreement with diode and film data. When compared to unfocused beams (ie, the focusing magnet removed), peak-to-entrance depth dose ratios were 11 to 14 % larger (depending on presence or extent of modulation). However, when using a more relevant comparison with beams collimated with an elliptically shaped collimator, the peak-to-entrance ratios were 26 to 38 % larger and per particle efficiency was ~ 2 fold greater than the later collimated beams.

Conclusion: Our results suggest that the use of rare earth magnet focusing assemblies is feasible and could improve dose-sparing of normal tissue and organs at risk while delivering enhanced dose to small elongated targets. Such magnets are small, inexpensive, do not require power or cryogenic cooling, have low external magnetic fields and could be incorporated in place of the aperture in a standard proton treatment nozzle.

#F-08

Impact of Combined Exposure to Anti-Orthostatic Tail Suspension and Low-Dose/Low-Dose-Rate Radiation on Hematological Parameters

Michael J. Pecaut, Daila S. Gridley, Mary Campbell-Beachler, Peter Gifford & Xiao Wen Mao

Department of Basic Sciences, Division of Radiation Research

The spaceflight environment consists of several factors including microgravity, low-dose/low-dose-rate radiation and psychological/physiological stress. Studies have shown that each of these environmental factors alone can influence central nervous system function and results suggest that deficits involve

neuroinflammation. A critical component of neuroinflammation is circulating immunocytes. Although previous ground-based studies are important, they rarely systematically investigate the interactions between these environmental factors.

We have initiated a series of ground-based studies using anti-orthostatic tail suspension in combination with LD/LDR gamma-radiation exposure. Briefly, whole-body gamma-irradiation was delivered to 6-month old C57BL/6 mice. A subset of these mice also underwent anti-orthostatic tail suspension. Mice were suspended and/or irradiated for 21 days. Seven days after completion of this period, mice were euthanized. Blood was collected via cardiac puncture and analyzed.

Although there were no significant main effects of either parameter on the count of any major immune subset, there were trends for suspension x interactions for white blood cell (WBC), lymphocyte and granulocyte counts. In all cases, this was due to decreases noted in the suspension alone or radiation alone groups that was not present in the combined treatment group. While there were no effects of radiation on the proportions of each population, there was a suspension-induced proportional shift away from lymphocytes toward granulocytes.

While there were overall suspension-induced decreases in erythrocyte count and hemoglobin levels, these decreases primarily occurred in mice that also received radiation, resulting in significant suspension x radiation interactions. A similar interaction was noted in hematocrit. There were also suspension-induced increases in both mean corpuscular volume and RBC distribution width, with a decrease in mean corpuscular hemoglobin concentration. There was only a trend for a radiation-induced increase in mean corpuscular hemoglobin. There was no impact of either stressor on platelet count or volume.

#F-09

Silver–gold alloy bimetallic nanoparticles as a therapeutic strategy against anaerobic pathogens

Megan S Holden, Jason Black, Marie-Clair Boutrin, Leroy G Henry, Francis Roy, Ozioma Chioma, Alexia Ximines, Wilson Aruni, Hansel M Fletcher, Christopher C Perry

Department of Basic Sciences, School of Medicine

We synthesized silver-gold (AgAu) bimetallic nanoparticles (NPs) via the galvanic replacement reaction between maltose coated silver NPs and HAuCl4 in 1-5% (w/v) triblock F127 copolymer solutions. This synthesis method is facile, economic, and environmentally benign. Silver NPs are commonly used as antimicrobials but their antimicrobial efficacy is limited typically to aerobic conditions that produce silver ions (Ag+). Because of this, silver NPs are ineffective against anaerobes. In the case of AgAu alloy NPs, the difference in redox potentials between Ag (~0.8 V) and Au(III) (~1.5V) results in surface sequestration of Ag+ ions that can be released into the solution under anaerobic conditions. We hypothesized that our synthesized AgAu NPs would exhibit antimicrobial activity against the anaerobic oral pathogen P. gingivalis. To test this hypothesis, we evaluated the effect of AgAu NPs on P. gingivalis planktonic growth rates, biofilm formation, and biofilm removal. AgAu NPs significantly inhibited P. gingivalis planktonic growth and biofilm formation. This inhibition was enhanced in the presence of hydrogen peroxide. Additionally, AgAu NPs significantly promoted P. gingivalis biofilm removal. These results suggest that AgAu NPs may be a promising therapeutic strategy against anaerobic pathogens.

#F-10

In cancer cell lines undergoing epithelial-mesenchymal transition, Snail expression correlates with downregulation of the tumor suppressor microRNA let-7

Aleksey Sebryakov and Juli Unternaehrer

Loma Linda University, Department of Basic Sciences, Division of Biochemistry, Center for Health Disparities and Molecular Medicine

Cells undergoing reprogramming paradoxically upregulate the epithelial-mesenchymal transition (EMT) transcription factor (TF) Snail, and Snail binds the promoters of several let-7 family members consistent with direct regulation. We hypothesize that Snail represses let-7 transcription during developmental and cancer-related EMTs. In embryonic stem cells differentiating to the primitive streak fate, cells following the mesendodermal lineage undergo EMT during a gastrulation-like event. Cells isolated at the primitive streak stage express high levels of Snail and other EMT TFs, and we observe decreased let-7 expression in the Snail-expressing cells. In epithelial and cancer cell lines, stimulation with TG#F-beta or EGF causes morphological and gene expression changes consistent with EMT. In breast, pancreatic, and ovarian cancer cell lines, increases in Snail expression correlate with decreased let-7 expression. The inverse relationship between Snail and let-7 could play an important role in cancer metastasis: in ovarian cancer, Snail expression is higher, and let-7 is lower, in metastasis; both of these also correlate with shorter survival times. In addition to its role in directly causing EMT and affording cells with the migratory and invasive phenotype necessary for metastasis, our model places Snail at an important control point for initiation of dedifferentiation events normally held in check by let-7.

#F-11

Application of a Pixelated Silicon Detector to Proton Radiosurgery

Andrew J Wroe, Anthony Teran, Grant A McAuley, Jeannie Wong, Marco Petasecca, Michael Lerch, Anatoly Rosenfeld

LLUMC, San Diego State University, LLU, University of Malaya, University of Wollongong, University of Wollongong, University of Wollongong

Background: To evaluate the applicability of a pixelated silicon detector known as the dose magnifying glass (DMG) to small field proton dosimetry and compare its performance against commercially available metrology techniques and Monte Carlo.

Methods: Proton radiosurgery requires metrology apparatus that have a high spatial resolution and a stable (or well characterized) response to LET. To meet this need the DMG was developed at the University of Wollongong. This device is a pixelated silicon strip detector comprising an array of 128 phosphor implanted n+ strips on a p-type silicon wafer. A 100 micron pitch device was tested at Loma Linda University Medical Center with proton radiation energies associated with radiosurgery applications. For these tests the DMG was mounted in a water tank with depth dose and lateral profiles collected simultaneously using the linear sensitive volume array for comparison with commercial ion chamber and diode detectors as well as Geant4 simulation results. The DMG response to varying dose rate was also evaluated and reported.

Results: The DMG performed well in these tests, providing real-time depth dose and lateral profile information. When compared with data from commercial measurement systems and Monte Carlo, agreement as a function of depth was very good. These results indicated that the device is largely LET independent in its response to proton radiation indicating potential for deployment in this radiation modality. Using the central channel of the DMG allowed for accurate point dose measurements without errors associated with partial volume sampling, while the full array could be used to provide real time profile information of fields below 1.5 cm diameter without the need for detector scanning. The DMG also exhibited a uniform response to dose rate up to 12 Gy/min, with under response noted for dose rates above this value.

Conclusion: The DMG is a useful device for proton therapy, in particular providing real-time data for small fields associated with proton radiosurgery applications. The device exhibited minimal variations in response as a function of LET and presents an interesting direction for development of real time dosimetry of both depth and lateral dose profiles. Future developments of the detector to provide larger linear detector arrays could widen the application of such a device to large field proton dosimetry and also beam characterization for pencil beam scanning applications. /

#F-12

Dose delivered to gantry-mounted electronics in proton therapy

Andrew J Wroe, Jerry D Slater

Loma Linda University Medical Center, Loma Linda University Medical Center

Background: To evaluate the scattered and secondary radiation fields present in and around a passive proton treatment nozzle to assess any electronic complications that may arise.

Methods: Landauer Luxel dosimeters were used to evaluate the radiation field around one of the proton passive scattering nozzles at Loma Linda University proton therapy center. These detectors use optically stimulated luminescence technology in conjunction with CR-39 to measure doses from X, gamma, proton, beta, fast and thermal neutron radiation. The dosimeters were stationed at various positions around the gantry pit and on racks on the gantry itself to evaluate the dose to electronics. Wax shielding was also employed on some detectors to evaluate the usefulness of this material as a dose moderator. To create the scattered and secondary radiation field in the gantry enclosure, a polystyrene phantom placed at isocenter and irradiated with 250 MeV protons to a dose of 1.3 kGy over 16 hours.

Results: The measured dose equivalent ranged from 100-6000 mrem, with proton/photon, thermal neutron, fast neutron and overall dose equivalent evaluated. The position of the detector/electronics relative to both isocenter and also neutron producing devices such as the collimators, first and second scatters definitely had a bearing on the dose received. Interestingly the addition of 1 inch thick wax shielding decreased the fast neutron component by almost 50%, yet this had a corresponding increase in thermal neutron dose of 100% and a 50% increase in photon/proton dose as there was no B-10 component to capture thermal neutrons.

Conclusions: The data obtained in this study will benefit future upgrades and facility designs by identifying mounting positions for electronics that minimize radiation dose.

#F-13

Evaluation of Standard Beam Delivery Devices in Proton Intracranial Radiosurgery

Andrew J Wroe, Jared Webster, David Bush, Jerry D Slater

LLUMC, LLU, LLUMC, LLUMC

Background: To evaluate the use of standard apertures and range shifters for the treatment of brain metastasis in proton stereotactic radiosurgery.

Methods: Five localized brain metastasis patients previously treated using our intracranial proton stereotactic radiosurgery procedure (i.e. with a custom aperture and bolus), were randomly selected from our patient cohort. The custom aperture and bolus treatment plans were used as the standard of care in this case and comparative treatment plans using the standard aperture and range shifter concept were generated. Gantry/table angle and the number of treatment beams were optimized as part of this study to evaluate the ability of the standard aperture/range shifter system to deliver a comparable treatment to the patient. Conformity index, homogeneity index, isodose volumes and integral dose were all evaluated to determine the degree of conformity of the plans created and for comparison to the custom aperture/bolus treatment modality.

Results: The generated treatment plans demonstrated that the standard aperture and range shifter combination could be used to produce comparable conformity index and isodose volumes to the custom aperture/bolus case in four out of the five patients studied. In two of the patients a comparative conformity index was achieved by optimizing the angles of the 3 treatment beams, while in two of the cases 1 or 2 additional beams were required. Additionally, this system exhibited efficiency gains over the custom aperture bolus system in reducing the time necessary for treatment planning, device manufacture and QA.

Conclusion: This work demonstrated that largely spherical shape of brain metastasis makes this target well suited to an application of standard apertures, while additionally providing efficiency gains in device manufacture and QA for treatment.

OTHER

#O-01

Prediction of small molecules targeting cancer stem cells

Curtis Younger and Ubaldo Soto

LLU

Prediction of small molecules targeting cancer stem cells / Curtis Younger and Ubaldo Soto / Division of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, CA 92354, USA / Cancer stem cells (CSC), a small subpopulation of cancer cells within a tumor that have stem cell characteristics, are known to be a key component in tumor initiation and cancer development. CSCs are resistant to standard chemotherapeutic drugs, therefore the identification of new chemicals targeting this cell population is imperative. Previous work in our lab has identified that the small molecule, aminoflavone, is efficient in

targeting breast cancer stem cells by inhibiting the expression of the cell-surface protein Integrin alpha-6 (A6IT). In that work, we also identified a gene expression pattern responsible for A6IT depletion, consisting of inhibition of c-myc and induction of fra-1 expression. We hypothesize that other small molecules inhibiting c-myc and inducing fra-1 will also be effective in targeting breast cancer stem cells. In this project, we tested Pyrrolidine dithiocarbamate (PDTC) to induce fra-1 expression, and 10058-F4 and 10074-G5 to inhibit ¬c-Myc protein activity. To test small molecule activity against MC#F-7 breast cancer stem cells, we measured the capacity of those molecules to disintegrate mammospheres, which are cell suspension cultures enriched in CSCs. In addition, we measured A6IT, c-myc, and fra-1 expression using RT-qPCR. We used FACS-analysis to confirm CSC population. We observed that PDTC indeed induced fra-1 and was able to disintegrate mammospheres in a concentration dependent manner. 10074-G5 was much more effective in disintegrating mammospheres than 10058-F4, despite the similarities in their molecular mechanism, which is inhibition of c-Myc by binding to its partner molecule MAX. 10058-F4 combined with PDTC was more effective than each molecule alone. In conclusion, we proved the concept that new small molecules can be identified to be effective in treating CSCs by imitating a similar molecular signature previously identified in targeting CSCs.

POSTDOCTORAL SCHOLAR

#O-02

IDENTIFICATION OF LMX1B REGULATED ENHANCERS IN LIMB MESODERM

Endika Haro Gabicagogeascoa, Luke Tegeler, Conor M. Spady, Emily J. Kim, Charmaine U. Pira. Kerby C. Oberg.*

Anatomy

Lmx1b is a LIM homeodomain transcription factor required for proper patterning during development. In the limb, Lmx1b expression is restricted to the dorsal limb mesenchyme and is responsible for limb dorsalization. Mice lacking Lmx1b function develop a ventral limb phenotype dorsally with footpads and ventral muscle flexors, whereas ectopic ventral expression of Lmx1b in the chick wing leads to the development of limbs with a double dorsal phenotype. Disruption of Lmx1b in humans is associated with Nail-Patella Syndrome and is characterized by nail dysplasia, absent or hypoplastic patellae, and progressive renal disease.

Despite the striking effect of Lmx1b on limb dorsalization, no direct targets in the limb have been described. In order to identify direct limb targets, a chromatin imunoprecipitation followed by massive parallel sequencing (ChIP-seq) was performed. Sites of Lmx1b binding were further analyzed for conservation across divergent vertebrate species to enhance discovery of Lmx1b-associated regulatory regions. To determine direct targets, sites of Lmx1b binding were compared with genes differentially expressed in the presence of Lmx1b. Enhancer activity of conserved non-coding DNA regions (CNRs) with Lmx1b binding sites were analyzed by reporter assays using electroporation in chick embryos.

We identified 590 genomic sites of Lmx1b binding and defined the Lmx1b binding motif. Conservation was evident in 169 of the sites and 34 of these CNRs were associated with 37 genes differentially expressed by Lmx1b. We validated 3 CNRs and showed Lmx1b regulated activity in the developing chick limb. Interestingly, one of the Lmx1b regulated CNRs located 60Kb upstream of the Lmx1b gene is only active in the dorsal limb mesoderm, mimicking the Lmx1b expression pattern. Furthermore, Lmx1b expression is increased 5-fold in

the presence of functional Lmx1b. Thus, this CNR appears to be an Lmx1b-assciated maintenance sequence (LAMS), generating a positive sel#F-regulatory loop.

#O-03

Identification of Alpha-enolase from Immunoseroproteomic Profiling of Autoantibodies to Tumor Associated Autoantigens in African American and Caucasian Men with Prostate Cancer

Tino Wilson Sanchez, Kwame Agyeman, Saied Mirshahidi, Nathan Wall, Colwick Wilson, Susanne Montgomery, Carlos A. Casiano

Center for Health Disparities and Molecular Medicine, LLU School of Medicine

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer-related male deaths in the U.S. PCa is diagnosed more frequently in African American men (AA), with a mortality rate two-fold higher than other ethnicities. The current diagnostic test for PCa relies on the detection of prostate specific antigen (PSA). Although this minimally invasive test has high sensitivity, its limited specificity leads to false positives and unnecessary biopsies. This test may also fail to recognize PCa in men with low PSA levels, especially obese AA men. Since AA men are diagnosed with PCa at a younger age than Caucasian men (CC), they are more likely to have low PSA levels. There is a critical need to identify minimally invasive biomarkers to complement the PSA test, especially in high risk populations like AA men.

Immunoseroproteomics offers a minimally invasive approach to detect early malignant processes that trigger the production of autoantibodies to prostate tumor-associated antigens (TAA). These antibodies serve as "reporters" to tumorigenic events and can be assessed as potential diagnostic biomarkers relevant to tumor biology. One and two-dimensional gel electrophoresis of proteins from aggressive PCa cell lines were probed with AA (n=40) and CC (n=50) PCa patient sera and analyzed using mass spectrometry to profile the anti-TAA antibody repertoire in a given patient. The AA PCa patient sera showed significantly higher titer in reactivity than the CC cohort even when normalized with similar cancer stage. A band around 50kD was identified in several sera and subsequent analysis through mass spectrometry showed the sera recognized alpha-enolase. Alpha-enolase is a metabolic protein that aids in cancer proliferation in several cancers including prostate cancer. It is important in tumor formation, expansion, glucose metabolism, and shows promise to be an autoantigen biomarker candidate for cancer detection.

#O-04

Small molecules of HPV E6 can re-sensitize HPV+ cancer cells to apoptosis induced by TRAIL and chemotherapy drugs

Chung-Hsiang Yuan, Maria Filippova, John Krstenansky, and Penelope Duerksen-Hughes

Center for Health Disparities and Molecular Medicine

High-risk human papillomaviruses (HR-HPVs) are responsible for nearly all cases of cervical cancer, as well as approximately 30% of all head and neck cancers. Two vaccines, Gardisil (Merck & Co.) and Cervarix (GlaxoSmithKline), can prevent infection; however, those vaccines are prophylactic rather than therapeutic.

Therefore, therapeutic approaches are still needed for patients who already have, or will develop, HPV-mediated tumors. The HR HPV E6 oncogene is well-known for its ability to accelerate p53 degradation, resulting in resistance to intrinsic apoptosis, the major cell-death pathway triggered by chemotherapy. In addition, our laboratory has demonstrated that expression of E6 protects cells from extrinsic apoptosis by binding to and accelerating the degradation of 2 major players in this pathway, caspase 8 and Fas-Associated Death Domain (FADD). We proposed that using small molecules to block the interaction between HPV E6 and its cellular targets, such as E6AP and/or caspase 8/FADD, had the potential to re-sensitize HPV+ cells to apoptosis induced by the cancer-specific ligand TRAIL and/or chemotherapy drugs. We have now identified two chemical compounds, myricetin and 6,7-dihydro-1H-imidazo[4,5-c] pyridine-6-carboxylic acid (DIPC), that demonstrate significant and specific inhibition of E6/caspase 8 binding in vitro. Using SiHa cells as representative of HPV+ cervical carcinoma, we were able to use both myricetin and DIPC to re-sensitize cells to apoptosis induced by TRAIL as well as the chemotherapeutic agents, doxorubicin and cisplatin. Caspase 3/7 activity increased following the combined treatment, demonstrating that the increase in cell death occurs through the apoptotic pathway. Furthermore, we found that myricetin and DIPC increase the level of caspase 8 and p53 in SiHa cells, but not in the HPV- C33A cells. Overall, our findings demonstrate that these small molecules are able to bind to E6, release bound E6-targets, and thus sensitize HPV+ cells to apoptosis induced by TRAIL- and chemotherapeutic agents-.

RESIDENT

#O-05

REMOTE ISCHEMIC PRE-CONDITIONING CAN MODULATE THE SYSTEMIC RESPONSE TO VVS AND DECREASES SYNCOPAL EPISODES

Sameer Soliman, Devin McBride, Jiping Tang, Richard Applegate II

Loma Linda Department of Neuroscience, Loma Linda Department of Anesthesiology

Syncope involves transient sel#F-limited loss of consciousness and spontaneous recovery within minutes. Treatment options for VVS are few, and often disappointing with an author from a Cochrane Database Review stating “there is insufficient evidence to support the use of any of the pharmacological or pacemaker treatments for vasovagal syncope”.3 The goal of this study is to determine if remote ischemic limb preconditioning (RIPC) will lessen the effect of a vasovagal event and potentially offer a new, unique alternative treatment for people that suffer from VVS.

Adult male Sprague-Dawley rats were anesthetized using intraperitoneal ketamine/xylazine mixture. Sinusoidal galvanic stimulation (sGVS) was used as this achieves similar hemodynamic effects to VVS4. Blood was drawn both before and after syncope for catecholamine level measurement by ELISA. RIPC is produced by placing a tourniquet on the hindlimb (about 200 mmHg) for 10 minutes at a time followed by 10-minute breaks for 4 cycles on 5, 10, or 15 consecutive days in different groups. Following RIPC, syncope will again be induced using sGVS. The hemodynamic response, CBF and catecholamines will be measured before and after sGVS again to check for a blunted response.

Our results indicate a vasovagal event occurs with the initiation of sGVS. The average heart rate before sGVS was 216, falling to 177 ($p<0.05$) after sGVS. The mean pressure ($p<0.01$) and CBF fell during sGVS as shown in

the table. CBF was measured in terms of perfusion units, showing a trend to falling CBF with sGVS. The results from the RIPC portion will be done by December 2014.

It is hypothesized that RIPC will decrease the effect of this and thus potentially be translated to humans who suffer from vasovagal syncopal episodes. Data from the planned RIPC experiments (expected to be completed by December 2014) will be presented.

STAFF

#O-06

Antenatal Chronic Hypoxia and L-Type Ca2+ Dependent Contractility of Pulmonary Arteries from Fetal Sheep

Alexander Brunelle, Quintin Blood, Lawrence D. Longo, Sean M. Wilson

Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA

Recently we and others have shown that K+-depolarization induced pulmonary arterial (PA) contraction is due to the combined activation of L-type Ca2+ channels (CaL) and Rho kinase (ROK). In fetal sheep we demonstrated that CaL inhibition with nifedipine (NIF) reduced 125 mM K+-mediated contraction up to about one-half, with ROK contributing the remainder, and that the relationship between these two pathways was preserved following antenatal chronic hypoxia (CH). To better define the relationship between these pathways and the impact of antenatal CH, we tested the hypothesis that direct activation of CaL would elicit a NIF sensitive contraction and this contraction would be preserved following antenatal CH. This was examined using wire-myography approaches on small diameter PA isolated from normoxic (~700 m) and antenatal CH (3,801 m, for >100 days) late gestation fetal sheep. Our results show that direct activation of CaL with 1 μM FPL 64176 (FPL) elicited a contraction that was approximately 80-125% of the maximum K+-induced depolarization (TKmax) in normoxic and hypoxic PA. In both normoxic and hypoxic PA 10 μM NIF reduced FPL-induced contraction back to baseline. These data illustrate that NI#F-sensitive CaL-dependent contraction is preserved following antenatal CH and when compared to our previous work that direct CaL activation causes fetal PA constriction in a distinct way compared to K+-depolarization.

#O-07

Diffusion Tensor Imaging (DTI) in Hippocampal Grey Matter

Faisal Rashid1, Eli Kinne-Lang2,3, Jenny Molet2,3, Tallie Z. Baram2, 3

Affiliations: 1Department of Pediatric Research, Loma Linda University. 2Departments of Anatomy and Neurobiology and 3Pediatrics, University of California, Irvine

Enduring early-life stress (ES) is thought to evoke structural changes in the brain. Epidemiological data in humans suggest that adverse experiences occurring early in life are associated with increased risk for developing cognitive problems in adulthood. We used a powerful naturalistic model of ES in rodents during the first week of life that is based on the generation of fragmented and unpredictable patterns of maternal care. Adult rodents exposed to fragmented maternal care have been found to develop cognitive deficits associated with structural changes to the hippocampal pyramidal cells in middle age. To further investigate alterations within the hippocampus following early-life stress, we undertook high resolution magnetic resonance imaging (MRI). Diffusion Tensor Imaging (DTI) measures water mobility within the brain and can

provide structural information about alterations within the hippocampus, a vulnerable brain region. Adult rodents exposed to fragmented maternal care have been found to develop cognitive deficits associated with structural changes to the hippocampal pyramidal cells in middle age. We hypothesize that high resolution DTI of the hippocampus will identify structural decrements. DTI successfully identified alterations in directional water movement within the HPC following additional stressors in a model of unpredictable pattern of maternal care. These structural alterations within stressed adult rodents exposed to fragmented maternal care may underlie the cognitive deficits observed in this model. High resolution DTI allowed for analysis of structural decrements in the hippocampus and other brain regions.

#O-08

wbeam: A User Friendly Web-based Interface for Monte Carlo Proton Beam Computer Simulations

Kenneth Williams, Grany McAuley, James M. Slater, Andrew Wroe

Radiation Medicine

Monte Carlo computer simulation (MCCS) of the passage of protons through a patient treatment nozzle and allows an accurate prediction of the radiation dose deposited in normal and target tissues. This is useful in research, but because such simulations can provide a rigorous verification of planned dose distributions produced by treatment planning software, there are potential clinical benefits. However, technical barriers tend to limit the routine use of MCCS except for the case of skilled programmers. The purpose of this project is to provide an intuitive user interface to our existing MCCS system that will allow non-programmers to perform simulations of protons delivered in the treatment rooms at LLUMC.

wbeam is an intuitive user interface for our MCCS software allowing selection of relevant beam parameters for simulation using modern web technologies. The interface is split into multiple tabs that allow for varying levels of customization and flexibility: The 'General' tab contains default parameter sets chosen from e.g. discrete drop down menus. An 'Advanced' tab allows greater customization for experienced users. For example, users can create a custom secondary scattering foil from a file description. A 'Phase Space' tab allows for setup of simulations generated from phase space files (i.e., pre-recorded particle momentum, energy, and position at preset locations along the treatment nozzle) resulting in reduced simulation times. An 'Analysis' tab allows users to analyze results and create various types of plotted output and data. Finally, the system outputs a file that can be used to rerun the simulation. The ability to easily produce accurate simulations of radiation dose delivered to planning target volumes is expected to benefit both researchers and clinicians.