NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

SYMPOSIUM PROGRAM & ABSTRACTS

UF | UNIVERSITY of FLORIDA

College of Medicine

AUGUST 9, 2013
8:00 A.M.
HPNP Building
Room G-312

University of Florida College of Medicine
Office for Diversity and Health Equity

NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

The program's success is due largely to the active participation
of the Faculty mentors listed below:

Phil Arlen, Ph.D.	Pandora Genomics, LLC
Kevin Brown, Ph.D.	Molecular and Cell Biology
Michael Clare-Salzler, M.D.	Department of Pathology
Kirk Conrad, MD	Department of Physiology & Functional Genomics
Nancy Denslow, Ph.D.	Biochemistry and Molecular Biology
Sylvain Dore, Ph.D.	Center for Translational Research in Neurodegenerative Disease
Linda Hayward, Ph.D.	Department of Physiological Sciences,
Nancy Hardt, M.D.	Department of Pathology and ObGyn
Jill Herndon, Ph.D.	Department of Health Outcomes and Policy
Maureen Keller-Wood, Ph.D.	Department of Pharmacodynamics
Bryan Kolaczkowski, Ph.D.	Department of Microbiology and Cell Science
Eric Krause, Ph.D.	Department of Pharmacodynamics
Mark Segal, M.D., Ph.D.	Department of Medicine, Division of Nehprology
Elaine M. Sumners, Ph.D.	Department of Pharmacodynamics
Charles E. Wood, Ph.D.	Department of Physiology & Functional Genomics

The Short-Term Research Training for Minority Students program is funded by a grant from the National Institutes of Health, National Heart, Lung and Blood Institute and supported by the University of Florida College of Medicine. The grant's principal investigator, Charles E. Wood, Ph.D., is the Professor and Chair for the Department of Physiology at the University of Florida.

STUDENTS	AFFILIATION
Natasha Berryman	Washington College
Joanne Bartley	University of Florida
Deina Bossa	University of Florida
Daniella Badal	University of Florida
Lily Garcia	University of Florida
Vanessa Obas	University of Florida
Mahogany Oldham	Malone University
Leshawn Richards	University of Florida
Brandi Thomas	University of Florida
Soweto Thomas	University of Maryland
Michelle Uzor	University of Georgia
Emiliano Valle	Harvard University
Keith Walters	Georgia Southern University
Simeon Walton	University of Florida

SYMPOSIUM PROGRAM

BREAKFAST AND WELCOME 8:00am

Charles Wood, Ph.D., Professor and Chair for the Department of Physiology

Michelle E. Jacobs, M.D., Assistant Dean, Office for Diversity and Health Equity
Professor, Department of Psychiatry, College of Medicine

SESSION I

SESSION CHAIR: Charles E. Wood, Ph.D.
Professor and Chair

Student: Simeon Walton 8:30am – 8:40am
Project: ***TRANSCRIPTION FACTOR FOXA2 IS A POSSIBLE CANDIDATE FOR THE PATHOGENESIS IN BREAST CANCE.***
Mentor: Kevin Brown, Ph.D.

Student: Emilano Valle 8:45am – 8:55am
Project: ***FROM CHILDHOOD STRESS TO JUVENILE CRIME: A STUDY OF ADVERSE CHILDHOOD EXPERIENCES AND VIOLENT CRIME IN FLORIDA'S JUVENILE DELINQUENTS***
Mentor: Nancy Hardt, M.D.

Student: Deina Bossa 9:00am – 9:10am
Project: ***EXPLAINING STATE VARIATION IN MEDICAID EXPENDITURES***
Mentor: Jill Herndon, Ph.D.

Student: Keith Walters 9:15am – 9:25am
Project: ***PDK4 AND MT-ND1 EXPRESSION IN FETAL SHEEP HEARTS IN RESPONSE TO INCREASED MATERNAL CORTISOL CONCENTRATION***
Mentor: Elaine Sumners, Ph.D.

Student: Joanne Bartley 9:30am – 9:40am
Project: ***EFFECTS OF PROTEASOME INHIBITION ON SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR SECRETION FROM HUMAN CYTOTROPHOBLASTS***
Mentor: Kirk P. Conrad, MD, Ph.D.

Student: Daniella Badal 9:45am – 9:55am
Project: ***THE PHARMACOGENOMICS OF CISPLATIN-INDUCED PEDIATRIC OTOTOXICITY: A META-ANALYSIS***
Mentor: Philip Arlen, Ph.D.

Student: Vanessa Obas 10:00am – 10:10am
Project: ***CIRCULATING ENDOTHELIAL CELLS AS A POTENTIAL BIOMARKER OF VASCULAR HEALTH IN SICKLE CELL DISEASE***
Mentor: Mark Segal, MD, Ph.D.

Student: Soweto Thomas 10:15am – 10:25am
Project: ***GENOTYPING SUB-CONGENIC B6.NOD CHR. 1 MICE***
Mentor: Michael Clare-Salzler, MD

BREAK 10:25am– 10:45am

SESSION II

SESSION CHAIR: Michelle E. Jacobs, M.D.
Assistant Dean, Office for Diversity and Health Equity

Student: Lily Garcia 10:45am – 10:55am
Project: ***THE CONTRIBUTION OF ANGIOTENSIN TYPE 1A RECEPTORS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO NEURONAL ACTIVATION AFTER SYSTEMIC ANGIOTENSIN II.***
Mentor: Eric Krause, Ph.D.

Student: Mahogany Oldham 11:00am – 11:10am
Project: ***HSD2 AND NPFF EXPRESSION IN THE NUCLEUS SOLITARUS TRACT IN RESPONSE TO DOCA-INDUCED SALT APPETITE***
Mentor: Linda Hayward, Ph.D.

Student: Michelle Uzor 11:15am – 11:25am
Project: ***STEROID HORMONE-RELATED GENE-EXPRESSION IN FATHEAD MINNOW OVARIAN FOLLICLE CELL PRIMARY*** **CULTURES**
Mentor: Nancy Denslow, Ph.D.

Student: Leshawn Richards 11:30am – 11:40am
Project: ***INSIGHT INTO RIG-I-LIKE-RECEPTOR (RLR) RNA RECOGNITION AND IMMUNE SIGNALING***
Mentor: Bryan Kolackowski, Ph.D.

Student: Natasha Berryman 11:45am – 11:55am
Project: ***THERAPEUTIC BLOCKADE OF THE PROINFLAMMATORY EP1 AND FP RECEPTORS AFTER TRAUMATIC BRAIN INJURY***
Mentor: Sylvain Dore, Ph.D.

Student: Brandi Thomas
Project: ***THE EFFECTS OF KETAMINE ON THE IMMUNE RESPONSE IN THE FETAL OVINE KIDNEYS IN RESPONSE TO HYPOXIC HYPOXIA***
Mentor: Charles Wood, Ph.D. 12:00pm – 12:15pm

Lunch **12:15pm – 1:15pm**
HPNP Building, Room G-312

PRESENTATION OF THE CERTIFICATES **1:15PM – 1:30PM**

PHOTOGRAPHS IN THE ARB COURTYARD **1:30PM**

ABSTRACTS

The Pharmacogenomics of Cisplatin-Induced Pediatric Ototoxicity: A Meta-Analysis

Daniella Badal, Philip Arlen, Ph.D

Pandora Genomics

Background: Cisplatin is a common chemotherapeutic drug used in the pediatric population for treating neuroblastomas, osteosarcomas, brain and solid tumors. However, in treating such malignancies, patients are also faced with cisplatin's most debilitating side effect: ototoxicity. Cisplatin-induced ototoxicity is usually permanent, bilateral and irreversible. Clinical reports have shown some degree of hearing loss in 25-90% of patients treated with cisplatin depending on age and dosage regimen. This hearing loss can have a significant impact on children's future speech and language development.

Objective: To determine how pharmacogenomics can be used to predict the incidence of cisplatin-induced ototoxicity in children.

Methods: Journal articles from PubMed, International Pharmaceutical Abstracts (IPA) and ISI Web of Science were retrieved using key terms related to ototoxicity ("hearing loss") in combination with "cisplatin and "pharmacogenomics" and "children." Publications meeting the following criteria were included in the meta-analysis: (a) must be cohort study or random controlled trial, (b) must focus on children <25 years old, (c) must incorporate genetic information and (d) cisplatin must be the only chemotherapeutic drug used. Repeated articles and articles featuring combination or radiation therapy were excluded.

Results: Data were extracted and tabulated from six publications which included ten single nucleotide polymorphisms (SNPs) across five different genes. Using an online Practical Meta-Analysis Effect Size Calculator, we calculated the odds ratio (OR), 95% confidence interval (CI) and risk ratio (RR) for ototoxic patients carrying the mutant allele (SNP) compared to the wild type allele. The values for each gene and its corresponding SNP are as follows: *TPMT rs12201199* (OR= 8.1081, 95% CI: 3.6989-17.7734, RR= 1.5468), *TPMT rs1142345*(OR= 7.1359, 95% CI: 2.8264-18.016, RR=1.5029), *TPMT rs1800460* (OR=9.8039, 95% CI: 3.0246-31.778, RR=1.5282), *COMT rs4646316* (OR=1.6496, 95% CI: 1.1734-2.319, RR=1.224), *COMT rs 9332377* (OR=2.3008, 95% CI: 1.5178-3.4879, RR=1.2901), *ABCC3 rs1051640* (OR= 1.967, 95% CI:1.3204- 2.9302, RR=1.3475), *LRP2 rs 4668123* (OR= 1.3846, 95% CI:0.4515-4.2464, RR= 1.1748), *LRP2 rs2075252* (OR=2.805, 95% CI:1.2503-6.2816, RR= 1.4563), *LRP2 rs2228171* (all values undefined due to sample size), and *GSTM3* (OR=0.1474, 95% CI: 0.0155- 1.4056, RR= 0.2895).

Conclusion: Overall, children who are genotypic carriers for the SNPs on the TPMT gene are most susceptible to cisplatin-induced ototoxicity. Specifically, carriers of the TPMT polymorphism rs1800460 are 9.8039 times more likely to have cisplatin-induced ototoxicity than non-carriers. Given the likelihood of attaining hearing loss by the presence and detection of certain polymorphisms, physicians now have an upper hand in better recognizing and potentially avoiding cisplatin-induced ototoxicity in pediatric patients.

"EFFECTS OF PROTEASOME INHIBITION ON SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR SECRETION FROM HUMAN CYTOTROPHOBLASTS"

Joanne Bartley, Bertha Campo, and Kirk P Conrad MD
Department of Physiology and Functional Genomics, University of Florida

Preeclampsia (PE) is a potentially lethal condition characterized by high blood pressure and abnormal amounts of protein in the urine of pregnant women. PE is a leading cause of maternal and perinatal morbidity and mortality. Research suggests that PE results from incomplete trophoblast invasion of the maternal uterus; as a result, small arteries that supply the uterus are not remodeled sufficiently. Insufficient remodeling is thought to cause reduced blood flow to the placenta resulting in ischemia and ischemia-reperfusion injury; the latter cause placental hypoxia and formation of reactive oxygen species, respectively [1].

During PE there is increased expression of Hypoxia Inducible Transcription Factor-1 α (HIF-α), Vascular Endothelial Growth Factor Receptor 1 (Flt-1) and Soluble (s) Flt-1[2, 3] by the placenta. Normally HIF-α proteins are rapidly degraded in an oxygen rich environment by the proteasome. However in PE, there is a marked increase of the HIF-α protein due to placental hypoxia and increased reactive oxygen species. In addition, proteasomal activity is markedly inhibited in PE placenta [4]. As a result of HIF-α over expression, potent anti-angiogenic factors such as sFlt and soluble endoglin are also increased and secreted into the maternal circulation, causing endothelial injury and disease manifestations [5]. However, another potential explanation for enhanced secretion of sFlt is reduction in proteasomal degradation following endocytotic recycling [6, 7].

For this study we hypothesized that proteasome inhibition would increase secretion of sFlt-1 anti-angiogenic growth factors from human cytotrophoblast cells.

HTR-8SVneo cells were grown in 12-well plates (250,000 cells/well) with 1 mL of RPMI medium supplemented with fetal bovine serum (FBS). Following 48 hours of incubation, cells were switched to serum free media in MG-132 (a proteasome inhibitor; final concentration 0.5 μM) or vehicle, with or without Q-VD-Oph (a caspase inhibitor; final concentration 100 μM) or vehicle. One 12-well plate was exposed to a "normoxic" environment (ambient air), while the other to a hypoxic environment (1% O2) for 12 hours. The 12-hour time point was chosen to minimize proteasomal inhibitor induced apoptosis [8]. Following the 12 hour incubation, the media was harvested for sFlt-1 analysis by ELISA. Total cellular protein was determined using the DC Protein Assay, and was used to normalize sFt-1 data.

The initial data show that cells exposed to a "normoxic" environment (n=12 wells) without treatment yielded an sFlt-1 concentration of 0.212± 0.040 pg/mL. Cells treated with Vehicle (VEH) for both Proteasome Inhibitor (PI) and Caspase Inhibitor (CI), VEH for PI with CI, PI with VEH for CI, PI with CI, and PI alone yielded sFlt-1 concentrations of: 0.178± 0.021 pg/mL, 0.159± 0.031 pg/mL, 0.385± 0.087 pg/mL, 0.332± 0.075 pg/mL, and 0.557± 0.396 pg/mL respectively. Cells exposed to a hypoxic environment (n=12 wells) without treatment yielded an sFlt-1 concentration of 0.156± 0.038 pg/mL. Cells treated with VEH for both PI and CI, VEH for PI with CI, PI with VEH for CI, PI with CI, and PI alone yielded sFlt-1 concentrations of: 0.138± 0.029 pg/mL, 0.133± 0.024 pg/mL, 0.281± 0.077pg/mL, 0.339± 0.072 pg/mL, and 0.249± 0.124 pg/mL respectively.

Summary: The results suggest that proteasome inhibition leads to the increased secretion of sFlt-1, in a "normoxic" and hypoxic environment. Caspase inhibition does not appear to have a significant effect on overall sFlt-1 secretion, thus excluding the trivial explanation of enhanced shedding of microvesicles as a consequence of apoptosis. Indeed in two experiments where we pelleted microvesicles in the media by centrifugation, the sFlt-1 concentration in the media was the same as media that was not centrifuged. If proteasome inhibition does indeed increase the secretion of soluble anti-angiogenic receptors, therapeutic strategies designed to enhance proteasome activity may prove useful in treatment of diseases in which proteasomal inhibition and anti-angiogenic balance may be pathogenic such as PE and Alzheimer's [9, 10].

References:

1. Conrad KP. Emerging role of relaxin in the maternal adapations to normal pregnancy: implications for preeclampsia.*Semin Nephrol*. 2011. **31**: 15-32.

2. Rajakumar A, Brandon HM, Daftary A, Ness R, and Conrad KP. Evidence for the functional activity of hypoxia-inducible transcription factors overexpressed in preeclamptic placentae.*Placenta*. 2004. **25**: 763-769.

3. Rajakumar A, Jeyabalan A, Markovic N, Ness R, Gilmour C, and Conrad KP. Placental HIF-1 alpha, HIF-2 alpha, membrane and soluble VEGF receptor-1 proteins are not increased in normotensive pregnancies complicated by late-onset intrauterine growth restriction.*Am J Physiol.* 2007. **293**: R766-74.

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7. Murdaca J, Treins C, Monthouel-Kartmann MN, Pontier-Bres R, Kumar S, Van Obberghen E, and Giorgetti-Peraldi S. Grb10 prevents Nedd4-mediated vascular endothelial growth factor receptor-2 degradation.*J Biol Chem*. 2004. **279**: 26754-61.

8. Fu JJ, Lin P, Lv XY, Yan XJ, Wang HX, Zhu C, Tsang BK, Yu XG, and Wang H. Low molecular mass polypeptide-2 in human trophoblast: over-expression in hydatidiform moles and possible role in trophoblast cell invasion.*Placenta*. 2009. **30**: 305-12.

9. Ambrose C.T. Neuroangiogenesis: A Vascular Basis for Alzheimer's Disease and Cognitive Decline During Aging. Journal of Alzheimer's Disease 23.3: 773-788, 2012.

10. Oddo S. the Ubiquitin-Proteasome System in Alzheimer's Disease. Journal of Cellular and Molecular Medicine 122: 363-376, 2008.

Therapeutic blockade of the Proinflammatory EP1 and FP Receptors after Traumatic Brain Injury

Natasha Berryman

Background: Traumatic brain injury (TBI) is a form of acute brain trauma and can lead to severe injury or even death. A TBI can cause detrimental effects such as neural inflammation, edemas, and excitotoxicity, with a limited amount of treatment methods available. According to previous works, after a traumatic brain injury there is an up-regulation of prostaglandins, that are lipids derived from arachidonic acid and helps with inflammatory responses. It is believed that prostaglandin receptors cause an increase in intracellular Ca^{2+} levels and toxicity. The EP1 receptor plays a role in Ca^{2+} signaling but little is known on its role following a TBI. There is another prostaglandin called a $PGF_{2\alpha}$, which can cause neuronal damage after activating a G-protein coupled receptor, the FP receptor. Therefore, the FP and EP1 receptors were explored in our study in order to investigate whether an antagonist drug for these receptors will provide a neural protective means after a TBI.

Methods: The effects of intraperitoneal injections (IP) post treatment using SC-51089, and AL-8810 after TBI was explored. These compounds are selective antagonists of prostaglandin EP1 and FP receptors, respectively. Also, 17-Phenyl-PGE_2 was used as a selective agonist of prostaglandin for EP1 receptors. The study consisted of wild type (WT), FP receptors knockouts ($FP^{-/-}$) and EP1 receptor knockouts ($EP1^{-/-}$) mice using controlled cortical impact (CCI) model, with 3mm impact, velocity 3.5-6 m/s, depth 1-2 mm and dwell time 100-200 ms. The neurological deficits were assessed 24h and 48 h after TBI using NDS, grip strength test, and Digigait. Also, anatomical observations were observed of cortical lesions in the brain, edema, and after 48 h and 10 days immunohistochemistry Iba1 (microglia) and GFAP (astrocytes) was conducted.

Results: After, analyzing the anatomical data there were no differences between the WT and $FP^{-/-}$ mice. During, the quantification and the analysis of data the consequences of the CCI were neuronal loss, hematomas, and localized edema derived from the hippocampus. After, 48hrs of the CCI, the cortical lesion volume and hippocampal swelling were significantly different compared to the sham mice. After using AL-8810 as a treatment method even though there was no significant effect on cortical lesions there was a reduction in hippocampal swelling not significantly different from sham mice. Also, the drug treatment NDS improved 24 and 48 hrs. after injury and the grip strength decreased. The $FP^{-/-}$ mice had a lower hippocampal swelling but not NDS compared to WT mice. The immunohistochemistry showed after a CCI glial and microglial activation in the penumbra, hippocampus, and lateral nuclei of thalamus. This was due to the blockade and genetic deletion of FP receptors and no changes were observed in GFAP and Iba1 in sham animals. The drug had no additional effects on $FP^{-/-}$ mice on GFAP and Iba1 immunohistochemistry test. The $EP1^{-/-}$ mice using the SC-51089 antagonist and 17-Phenyl-PGE_2 agonist had a similar NDS to the mice with no treatment, showing no significant effect of the drugs on EP1 receptors.

Conclusion: This study provided insight on the roles of FP as a target of CNS drugs for the treatment of a TBI. However, further studies on the role of EP1receptors should be conducted to understand treatment measurements on TBI.

Explaining State Variation in Medicaid Expenditures

Déina Renée Bossa

Explaining State Variation in Medicaid Expenditures

Deina Bossa

Established as part of the Social Security Amendments of 1965, Medicaid provides healthcare coverage to America's low income families with children, pregnant women and disabled individuals.[1] There are vast differences in per capita Medicaid expenditures between states which may signify differences in access to services or inefficiencies. Nevada, the lowest spending state, has per capita Medicaid expenditures of $579 and New York, the highest spending state, has per capita expenditures of $2,763. States must adhere to federal guidelines, but they have flexibility in administering their Medicaid programs by establishing eligibility criteria, scope of benefits, and cost sharing practices.[2] This, however, only explains some of the observed variation in spending. Other factors contribute to variation in spending including those affecting the demand for Medicaid services, the availability of state revenues to fund Medicaid programs, the supply of providers available to render services, and the cost of providing services to Medicaid beneficiaries. This study expands upon existing knowledge of the factors associated with differences in Medicaid spending by using regression analysis to determine which factors are significant and have greater relative importance in explaining spending variation. Results indicate that per capita state revenue, number of physicians per 10,000 population, and the percentage of the population living in households with ≤139% of the federal poverty level (FPL) are significant in explaining spending differences. Holding all other studied factors equal, a 1% increase in the percent of the population that is up to 139% of the FPL leads to a $30.10 increase in per capita Medicaid expenditures. A $100 increase in per capita state revenue leads to a $6.90 increase in per capita Medicaid expenditures and a one physician increase in the number of physicians per 10,000 population leads to a $46.57 increase in per capita Medicaid expenditures. To address differences in access to services among Medicaid beneficiaries in different states, it is recommended that policies be enacted that will affect the percent of the population that is up to 139% of the FPL, the amount of resources available to states to fund Medicaid programs, and the number of physicians in each state. This is because these factors have greater relative importance in affecting spending variation and may therefore affect access to services.

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[2] Centers for Medicare and Medicaid Services. *Medicaid information by topic.* Retrieved July 2, 2013, from http://www.medicaid.gov/Medicaid-CHIP-Program-Information/By-Topics/By-Topic.html

The Contribution of Angiotensin Type 1a Receptors in the Paraventricular Nucleus of the Hypothalamus to Neuronal Activation After Systemic Angiotensin II.

Esther L. Garcia, Lei Wang, Justin A. Smith, Helmut Hiller, Annette D. de Kloet, and Eric G. Krause, PhD, Department of Pharmacodynamics, College of Pharmacy, University of Florida

Hypertension, more commonly known as high blood pressure, is a common condition that currently affects 67 million people in the US (CDC). Elevated blood pressure is a major risk factor for various cardiovascular problems, including heart attack and stroke. Five classes of drugs can be found in the market today to treat this "silent killer" condition. However, due to the lack of efficacy of our current drug therapy, up to 30 % of patients remain nonresponsive to treatment after multiple drug regimens, exhibiting a so-called resistant hypertension and a desperate need for higher specificity drug targets (Calhoun *et al*. 2008). One hypothesis that accounts for this type of hypertension is the neuroadrenergic hypothesis, suggesting that the disease originates in the brain. Furthermore, two areas of the brain that have been of interest to scientists are the subfornical organ (SFO), one of the circumventricular organs of the brain where there is an incomplete blood brain barrier, and the paraventricular nucleus (PVN) of the hypothalamus, which regulates cardiovascular function, stress responsiveness, and neuroendocrine systems. Previous research has suggested that systemic angiotensin II (ANGII), an endocrine factor heavily implicated in the development of hypertension and cardiovascular disease, increases blood pressure by activating Angiotensin Type 1a receptors (AT1R) in the SFO. It has also been suggested that neurons in the SFO release ANGII from efferents terminating in the PVN, which in turn, elevates blood pressure by stimulating AT1R expressed on neurons in this nucleus. To test this hypothesis, we conducted a preliminary study utilizing the cre-lox system in mice to selectively delete the AT1R from PVN neurons. Male mice were divided into four groups: wild type mice given control injections of isotonic saline (WT-CON), wild type mice given systemic ANGII, PVN AT1R knock-mice given isotonic saline, PVN AT1R knock-mice given ANGII. Ninety-minutes after the injections, mice were euthanized, perfused, and brains were extracted and cryoprotected in 30% sucrose. Subsequently, brains were sectioned coronally and processed for Fos immunohistochemistry, a marker on neuronal activation. Preliminary results found an increase in Fos induction within the SFO relative to mice given saline injections. However, there were no effects of ANGII or PVN AT1R knock-out on Fos induction in the PVN, which may be the result of elevated Fos induction after saline and a modest Fos response to ANGII. Follow-up studies will reduce handling stress associated with injections and adjust the dose of ANGII in an attempt to attenuate Fos induction following saline administration but augment Fos induction after ANGII.

References:

Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD,White A, CushmanWC,WhiteW, Sica D, Ferdinand K,Giles TD, Falkner B & Carey RM (2008). Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation* **117**, e510–e526.

CIRCULATING ENDOTHELIAL CELLS AS A POTENTIAL BIOMARKER OF VASCULAR HEALTH IN SICKLE CELL DISEASE

Vanessa Obas, Larysa Sautina, and Mark S. Segal

Division of Nephrology, Hypertension and Transplantation, Department of Medicine, University of Florida, Gainesville, Florida

Background: Patients with sickle cell disease (SCD) possess an inherited genetic mutation that promotes the aggregation of adult hemoglobin molecules and consequent sickling of the red blood cell. The distortion of the red blood cell shape promotes the major clinical hallmark of the disease: painful vaso-occlusive episodes. Hydroxyurea is currently the only FDA-approved medication demonstrated to manage the pain associated with SCD. There does not exist an objective marker of vascular health to guide the appropriate dosing of the medication. Research has shown that circulating endothelial cells (CECs) are markedly increased during instances of vascular damage. We hypothesize that CEC enumeration can be utilized to interrogate ongoing vascular damage in SCD patients, objectively dose hydroxyurea, and assess the efficacy of other treatments for SCD. **Methods:** The study population was made up of 1) healthy nonsmoking individuals, 2) SCD patients managing their disease with hydroxyurea, and 3) SCD patients managing their disease without hydroxyurea. The number of CECs was enumerated for each study group. **Results:** In control subjects, CEC numbers were low (< 12 CECs per mL). We predict that SCD patients will have elevated CEC numbers relative to control participants. The impact of hydroxyurea on ongoing vascular injury has not been previously studied. We predict that SCD patients using hydroxyurea will have diminished CEC numbers relative to their counterparts without the medication. RNA has been isolated to examine the expression pattern of proteins related to vascular injury in each study group. **Conclusion:** Circulating endothelial cells may be a useful tool to investigate ongoing endothelial injury in SCD patients and aid in the medical management of the disease. Future studies will investigate the expression of proteins related to endothelial damage (like VCAM-1) as biomarkers of vascular injury in SCD patients.

HSD2 and NPFF Immunoreactive Expression in Response to Sodium Diet

National Institute Of Health-Summer Research 2013

Physiology Department

Mahogany R. Oldham

Cardiovascular disease namely hypertension has evolved into a worldwide epidemic and the numbers of affected individuals steadily increase stemming from a wide variety of causes. Reports indicate that in the year of 2009, 348,000 Americans died from complications associated with high blood pressure. In the study of hypertension we focused on neuronal and humoral activation as it relates to sodium consumption. This study will interpret excess salt intake as an initiating response of HSD2 and NPFF expression, related to the stimulation of aldosterone release. Given that the 11-B HSD2 protein resides in close proximity with the neuropeptide FF (NPFF) peptide in the nucleus tractus solitarius, we hypothesized that NPFF expression has some correlation to salt intake and/or aldosterone activation. In the experiment, Sprague Dawley rats were used as a model. There were two groups of rats tested; Sprague Dawley rats and a group of Sprague Dawley rats implanted with a time release pellet (60 days) surgically inserted in their neck which slowly released deoxycorticosterone acetate (DOCA), the precursor to aldosterone, which makes the rats crave salt. When these DOCA rats are given access to saline solution they ingest large quantities. Salt and food intake typically occurs during the night in rodents so this is an important factor to take into consideration.

There were four subpopulations tested; 1. DOCA night rats, 2. DOCA day rats, 3. SD day rats. 4. SD night rats (The night and day represents when the rats were sacrificed, 9pm and 11am, respectively). We hypothesized that there would be higher activation of 11-B HSD2 and NPFF at night versus the day, and that these markers would be located within the same cells. This hypothesis was tested by evaluating gene expression in the medulla using real time PCR and counting HSD2 and NPFF positive cells stained by immunohistochemistry. Our results showed that there is a higher intake of salt in DOCA rats specifically at night rather than in the day. The Sprague Dawley rats (which were our control model) followed an AM/PM pattern of HSD2 and NPFF cell activity while the DOCA rats displayed less of an AM/PM variation. Based upon the 142 sections analyzed, the findings of our immunohistochemistry methods demonstrated that HSD2 and NPFF are not likely to co-exist within cells, contrary to our hypothesis. Real time PCR data suggested that HSD2 expression may play a less significant role in salt appetite, and may have a larger essential function which has not been discovered. Further data analysis is forthcoming.

INSIGHT INTO RIG-I-LIKE-RECEPTOR (RLR) RNA RECOGNITION AND IMMUNE SIGNALING

Leshawn Richards, Bryan Korithoski Ph.D., Bryan Kolaczkowski Ph.D.

Department of Microbiology and Cell Science

RIG-I-like receptors (RLRs) are a family of pathogen recognition receptors (PRRs) that are recognize viral RNA in the cytoplasm and initiate innate immune responses. The RLRs RIG-1 and MDA5 have been found to be required for the detection of numerous viruses. Aberrant RLR signaling or malfunctions of RLR expression are involved in susceptibility to viral infection and the development of auto-immune disorders. My project begins to explore which RNA molecules the RLR recognizes. It is known that RIG-I preferentially recognizes RNA sequences marked with 5' triphoshphorylated (5' ppp) ends, while MDA5 prefers long, double-stranded RNA and synthetic high-molecular weight poly (I:C) fragments. I tested the binding of an ancestor of MDA5 and LGP2 to synthetic RNA to provide insight into the evolutionary changes in RNA binding that have occurred. To do this, I started with synthetic DNA, and then generated 5' ppp ssRNA which I then dephosphorylated. The RNA was then biotinylated in order to measure the binding affinity to the ancestral construct using Bioforte's® OCTET machine. The results of my experiment will be used to analyze and compare the binding relationships between the RLR proteins.

The effects of ketamine on the immune response in the fetal ovine kidneys in response to hypoxic hypoxia

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Hypoxia is a decrease in oxygen supply delivered to the body. There are three subtypes of hypoxia during pregnancy. The first is pre-placental hypoxia, where both the mother and her fetus will be hypoxic caused by high-altitude or cyanotic maternal heart disease. The next type is utero-placental hypoxia, which is when the maternal oxygenation is normal but the utero-placental circulation is impaired due to events such as preeclampsia or placental insufficiency. Finally, there's post placental hypoxia, where only the fetus is hypoxic. A cause of pre-placental hypoxia is hypoxic hypoxia, a mild form of fetal stress where the maternal and fetal partial pressure of oxygen is decreased via decrease in maternal ventilation. We hypothesized that hypoxic hypoxia causes an immune reaction in the fetal ovine kidneys, and ketamine, an NMDA receptor antagonist primarily used for anesthesia, decreases lymphocytic infiltration. We examined fetal ovine kidneys where mother and baby were chronically catheterized, and tracheostomy was performed on the ewe. Fetal hypoxic hypoxia, a 50 % decrease in partial pressure of oxygen, was induced by administering nitrogen gas directly to the ewe for 30 min. Ketamine was administered intravenously to the fetus 10 min prior to the induction of hypoxia. Kidneys were collected 24 hours post hypoxic stimulus, fixated in 4% paraformaldehyde, and then embedded in paraffin blocks for hematoxylin and eosin (H&E) staining. H&E staining is used in histology to visualize the tissue's morphology. After tissue examination under light microscope, lymphocytes and neutrophils were observed in the hypoxic kidneys. Also, tissues treated with ketamine showed very little or total absence of immune cells. Further immunohistochemistry studies using CD5 marker, a lymphocyte B and T surface antigen, will be conducted to quantify lymphocytic expression. We conclude that ketamine decreased the presence of lymphocytic infiltration in the fetal ovine kidney exposed to hypoxia.

Soweto Thomas

8/1/13

Dr. Clare-Salzler Lab

Genotyping Sub-congenic B6.NOD Chr. 1 Mice

Type 1 Diabetes (T1D) is a multifactorial, autoimmune disease, caused by self-destruction of the β cells in the pancreas. With T1D, β cells release antigens which are taken by APCs through Pathogen Recognition Receptors (PRR). APCs are then activated by PRR, and induce secretion of IFN-α/β. IFN-α/β, associated with infection, induces the attack of T-cells on β cells, resulting in cell death, lack of insulin production, and in turn hyperglycemia. Using a T1D diabetic mouse model called non-obese diabetic (NOD), previous studies in the lab have shown that IFN-α/β is present at higher levels in these NOD mice than in control mice. These studies have also linked IFIH1 alleles to T1D, implicating a genetic link between IFN-α/β and T1D progression.

Building upon these findings, congenic mice with specific NOD chromosomes were used to find the source of the increased and sustained IFN-α/β responses. B6.NOD Chr. 1 mice were shown to exhibit the most similar IFN-α/β responses to the regular NOD mice. The next step was to find which genes on the chromosome were specifically responsible for the IFN-α/β response. Sub-congenic mice were bred with various specific portions of Chromosome 1 to further narrow down the source of the IFN-α/β response.

We are currently in the process of genotyping B6.NOD Chr. 1 mice to guarantee they have only the intended sections of B6.NOD Chr. 1. We performed PCR on the DNA of different sub-congenic B6.NOD Chr. 1 mice (C1C, C1CB, C1CP), running them against different markers specific to regions on chromosome 1. Identifying the genotypes of these mice ensures they were bred properly, so in future studies we can monitor interferon responses and attribute them to the portions of NOD chromosome 1 that they carry.

Steroid Hormone-Related Gene Expression in Fathead Minnow Ovarian Follicle Cell Primary Cultures

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Background and Significance: The fathead minnow plays an essential role as a fish model in laboratory experiments pertaining to aquatic toxicology. Its small size and high fecundity make the fathead minnow an ideal tool for testing water quality and reproductive responses to contamination. Primary cell cultures serve as the best representative of the main functional components of the tissue from which they are derived and retain many of the characteristics of the cell *in vivo*. The follicle cells, located within the ovary, are an important component of female reproductive biology. Aromatase, the enzyme responsible for the biosynthesis of testosterone to estrogen, is expressed in the follicle cells. The mRNA expression of aromatase is known to be lost over time in culture, but no study has shown when this takes place in this species. Upon successful completion of characterization, follicle cell cultures can then be used in other studies to measure the effects of various contaminants on multiple aspects of reproductive physiology.

Hypothesis: Aromatase mRNA expression levels will decrease over time, but cells stimulated with human chorionic gonadotropin (hCG) will maintain expression for a longer period of time than cells in non-stimulated environments.

Methods: Reproductively mature female fathead minnows were anaesthetized with MS222 and ovaries were removed immediately after. Isolation of follicular cells from oocytes is based off of the protocol developed by (The Thomas Lab, University of Texas) with minor modifications. Briefly, ovaries were minced and washed in L15 media (5% ABAM) before digestion with 0.0125% Trypsin (1% ABAM). Digested tissue was then passed through a 100um cell strainer to separate out the oocytes. Follicle cells were then subjected to two washes followed by removal of the red blood cells by centrifugation over a Percoll cushion. Purified follicle cells were then washed and counted before plating. Cells were then cultured in L15 supplemented with 1% ABAM and 2% charcoal stripped fetal bovine serum. Two test groups were created: one treated with hCG (10 I.U./mL, treatment starting after day 2 and 2) without hCG. Cells were plated at a concentration of ($1x10^6$ cells/ well). Samples were collected at day 0, day 2, and day 4. At day 4, cell viability was measured using the AlamarBlue assay, placed in 0.75mL STAT-60, and kept in -80 until RNA extraction. Culture medium was collected every day and saved for further analysis of E2 production. Total RNA was extracted was converted to cDNA using reverse transcriptase. Primers were then obtained and qPCR was conducted to analyze mRNA expression of aromatase.

Results: Analysis of AlamarBlue cell viability assay showed no significant differences between groups treated with human chorionic gonadotropin and control. Following qPCR analysis for the expression of the CYP19A and 18S housekeeping gene, it was determined that further optimization of the procedure is required for relative quantitation using the ΔΔCT method. Further work will focus on determining time course for aromatase gene expression and whether stimulated environments effect the overall expression of various genes found in the ovarian follicle cell.

"FROM CHILDHOOD STRESS TO JUVENILE CRIME: A STUDY OF ADVERSE CHILDHOOD EXPERIENCES AND VIOLENT CRIME IN FLORIDA'S JUVENILE DELINQUENTS"

Emiliano Valle

The study of Adverse Childhood Experiences (ACEs) has attracted increased attention since the topic was first introduced15 years ago. It is clear from the vast research on the subject that ACEs have tangible negative effects on adult health and behavior. However, studies on the effects of ACEs on adolescents are scarce. This investigation examines possible relationships between violent crime and ACEs in 64,329 youth in the Florida juvenile justice system from 2007 to 2012. Violent crime is a cause of great concern in communities and this study attempts to improve understanding of what ACEs might contribute to its occurrence in Florida's youth. The Positive Achievement Change Tool (PACT) assessment, which is administered to youth in Florida's juvenile justice system, reveals what types of ACEs each youth has encountered during his or her childhood. Coupling these data regarding ACEs with a youth's offenses allowed for analysis of two groups: youth who committed at least one against-person felony (violent crime) and youth who did not. Analyses of these groups indicated that a greater percentage of youth who committed against-person felonies had high ACE composite scores (ACE sums) compared to youth who did not. Additionally, certain types of ACEs were more prevalent in against-person felons than in all other juvenile delinquents. These findings imply that while action should be taken to reduce incidents of all ACEs, when attempting to lower the rate of violent crime, certain ACEs should be primary targets.

PDK4 and MT-ND1 expression in fetal sheep hearts in response to increased maternal cortisol concentration

Keith Walters

Recent past studies have both displayed and confirmed that increases in the plasma cortisol levels of maternal sheep hearts during the period of late gestation result in fetal heart enlargement (Reini et al. (2008). In this study, we analyzed the change in gene expression for the enzymes MT-ND1 and PDK4 in the fetal heart in an attempt to find a possible correlation between their level of expression and fetal heart enlargement. Past microarray experiments suggested that there would be an increase in the expression of PDK4 and MT-ND1 in response to increased maternal cortisol concentration. This increase in expression would establish a consistency with the change in the energy production pathway of the fetal heart. The complementary DNA (provided through reverse transcriptase of sheep RNA) from the septum of fetal sheep delivered from mothers both infused with cortisol and not infused with cortisol were extracted and analyzed by quantitative real-time PCR. The fetal sheep from which the DNA was retrieved were also categorized by the length of exposure to increased maternal cortisol which began at day 115 of gestation and continued until 130 days and 140 days. Since cortisol matures fetal organs, this categorization could of also helped determine if PDK4 and MT-ND1 had a role in increasing the rate of fetal heart development. These two groups were then both split into an experimental (cortisol-treated) group and control (saline-treated) group. The fetal sheep cDNA was placed into a PCR reaction mixture that included the following components: nuclease free H20, a forward primer, a reverse primer, and a Taqman/SYBR green polymerase reagent to enable duplication of the cDNA. The PCR test results only showed statistical significance ($p<.05$) for 140 day vs. 130 day controls and no significance for 130 day cortisol vs. 130 day control groups or the 140 day cortisol vs. 140 day control groups. There seemed to be no significant change in expression of these genes between the experimental and control groups. These findings suggest that MT-ND1 and PDK4 expression in the fetal heart may not have a valid relation with increased cortisol concentration in the pregnant maternal sheep. Therefore, the exact mechanism behind cortisol's effect on the fetal heart and its enlargement remains unknown.

TRANSCRIPTION FACTOR FOXA2 IS REQUIRED FOR THE MAINTENANCE OF MESENCHYMAL PHENOTYPE IN MDA-MB-231 BREAST CANCER CELLS.

Simeon Walton

FOXA2 is a member of the forkhead family of transcription factors and is linked to cell differentiation during embryonic development. Several groups have shown that FOXA2 is essential in the suppression of the epithelial-to-mesenchymal transition (EMT) in pancreatic and lung cancer; however, whether FOXA2 is a candidate in the pathogenesis in breast cancer is unknown. Using expression microarray, our lab observed that FOXA2 expression requires the Ataxia Telangiectasia, Mutated (ATM) kinase tumor suppressor and I confirmed this finding using Q-PCR; however, the molecular mechanism behind this observation remains unknown. NF-kB is a transcription factor activated by ATM and using the established NF-kB inhibitor BAY-117082, I tested if NF-kB regulates FOXA2 expression. I also found that only MDA-MB-231 cells express FOXA2 when a panel of breast cancer lines were tested by Q-PCR. To examine the role of FOXA2 in MDA-MB-231 cells I used Lentivirus-encoded shRNA to decrease expression of FOXA2 in this cell line. FOXA2 knockdown in this line appeared to produce a morphological phenotype in this cell line reminiscent of a transition of the normally mesenchymal phenotype of this cell line to an epithelial cell phenotype (ie, MET). To further examine this, Q-PCR was conducted to examine expression of the mesenchymal marker genes Vimentin, N-cadhedrin, and Fibronectin, and the epithelial line EPCAM. Finally, the motility of FOXA2 knockdown MDA-MB-231 was examined using a scratch-wound assay.