

2009 Southeast IDeA Regional Meeting

Proceedings

Charleston, South Carolina November 9-11, 2009

2009 Southeast IDeA Regional Meeting

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AGENDA

Monday, November 9, 2009

4:00 p.m.	Colonial Ballroom	Registration
5:00 p.m.	Colonial Ballroom	Welcome <i>Lucia Pirisi-Creek</i>
5:15 p.m.	Colonial Ballroom	NCRN and IDeA Overview <i>Michael Sayre</i>
5:45 p.m.	Colonial Ballroom	Plenary Speaker <i>Vladimir Mironov, "Organ Printing: How to print a human organ"</i>
6:30 p.m.		Dinner on Your Own

Tuesday, November 10, 2009

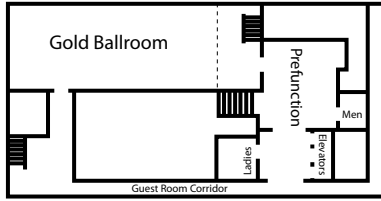
7:00 a.m.	Gold Ballroom	Poster Set-up
7:30 a.m.	Colonial Ballroom	Registration and Continental Breakfast
8:30 a.m.		Scientific Session I <i>Neuroscience I - Colonial Ballroom</i> <i>Cancer Research - Calhoun Ballroom</i> <i>Biochemistry - Pinckney Ballroom</i>
10:00 a.m.		Break
10:30 a.m.		Scientific Session II <i>Neuroscience II - Colonial Ballroom</i> <i>Microbiology/Immunology/Inflammation - Calhoun Ballroom</i> <i>Bioinformatics - Pinckney Ballroom</i>
12:00 p.m.	Gold Ballroom	Lunch
1:00 p.m.	Gold Ballroom	Poster Session
3:00 p.m.	Colonial Ballroom	IDeA-CTSA Collaborations: Opportunities and Challenges <i>Organizer: Sidney McNairy</i> <i>Chair/Moderator: Fred Taylor</i> <i>Panelists: Kathleen Brady, Lawrence Cornett, Jeff Ebersole, Anthony Hayward, Curtis Lowery, Elizabeth Ofili, Lucia Pirisi-Creek, Thomas Ziegler</i>
4:45 p.m.		Break
5:00 p.m.		Scientific Session III <i>Neuroscience III - Colonial Ballroom</i> <i>IDeA Implementation/Outcomes - Calhoun Ballroom</i> <i>Regenerative Medicine - Pinckney Ballroom</i>
6:30 p.m.	Colonial Ballroom	Reception and Award Presentation
7:00 p.m.		Dinner on Your Own

Wednesday, November 11, 2009

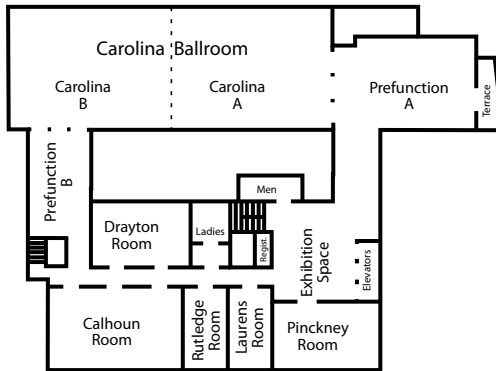
7:00 a.m.	Colonial Ballroom Calhoun Ballroom	Continental Breakfast Executive Session <i>IDEA Pls with NCCR/DRI Staff (breakfast included)</i>
8:00 a.m.	Colonial Ballroom	Diversity in Science Panel Discussion <i>Panelists: Anthony DePass, Shawn Drew, Sandra Glover, T. Scott Little, Judith Salley, John Wheeler, Cynthia Wright</i>
9:30 a.m.	Colonial Ballroom	Shared Facilities and Resources: NICL, NAIPI Tools - General Discussion <i>Panelists: W. Scott Argraves, Konstantin G. Kousoulas, Lucia Pirisi-Creek</i>
10:30 a.m.		Break
11:00 a.m.	Colonial Ballroom	The Future of Cyberinfrastructure and Communication - General Discussion <i>Karin Remington</i>
12:30 p.m.		Break (Boxed Lunches Available)
12:45 p.m.	Colonial Ballroom	NIH Opportunities in Biotechnology, Commercialization, and Clinical Research Education <i>Sidney McNairy, Opening Remarks</i> <i>Douglas M. Sheeley, "Resources Available Through the Biomedical Technology Research Centers"</i> <i>Lili M. Portilla, "Commercialization and the SBIR/STTR Programs"</i> <i>David Wilde, "Clinical Research Education and Resources"</i>
1:45 p.m.		Meeting Adjourned



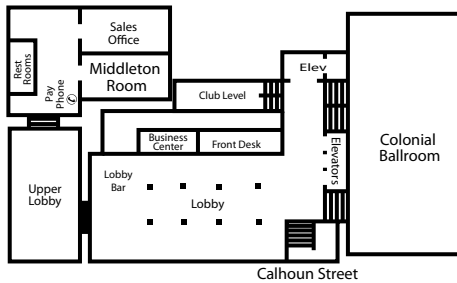
FRANCIS MARION
HOTEL



The Gold Ballroom/Second Floor



Mezzanine/Meeting Level



The Colonial Room/Lobby Level

ABSTRACTS

Biochemistry

BC-O1. CNS Gaz subunit proteins are essential to inhibit high salt-induced increases in AVP secretion, water retention and hypertension

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We have previously shown that brain Gaz and Gaq protein-gated pathways have opposing inhibitory and stimulatory, respectively, effects on vasopressin (AVP) release evoked by pharmacological and physiological stimuli (Am. J. Physiol, 295: R535-542, 2008). These studies tested the hypothesis that intact central Gaz protein pathways are required to appropriately regulate AVP secretion, total body water balance and blood pressure during a chronic high salt challenge in salt-resistant rats. Methods Sprague-Dawley (SD) rats, which are known to be salt-resistant, were maintained on control (NS) or 8% NaCl (HS) chow for 3 weeks. At the end of the treatment period, metabolic balance studies and blood pressure measurements were performed and plasma AVP, and Gaz and Gaq protein levels in different brain regions were quantified. These parameters were also assessed in separate groups of SD rats fed NS or HS chow during chronic (21-days) intracerebroventricular (i.c.v.) miniosmotic pump infusion of a Gaz or scrambled (SCR) oligodeoxynucleotide (ODN). Results: In SD rats, chronic HS intake did not alter basal plasma AVP levels or blood pressure. However, as compared to rats fed NS, HS intake produced an endogenous and marked down-regulation of Gaq (8-fold; $P < 0.05$), but not Gaz protein levels, selectively in the hypothalamic paraventricular nucleus (PVN). In other studies, in SD rats maintained on HS intake, chronic down regulation of central Gaz proteins via continuous i.c.v. Gaz ODN infusion caused profound increases in basal plasma AVP levels (AVP [pg/ml]; HS + SCR ODN, 1.38 ± 0.2 vs. HS + Gaz ODN, 4.6 ± 0.3 , $P < 0.05$), total body water balance (24-h H₂O balance [ml/day]; HS+ SCR ODN, 15 ± 3 vs. HS + Gaz ODN, 42 ± 4 ; $P < 0.05$) and mean arterial blood pressure (MAP [mmHg]; HS + SCR ODN, 128 ± 2 vs. HS + Gaz ODN, 152 ± 3 , $P < 0.05$). Compared to naive SD rats, SCR ODN infusion did not alter any parameter demonstrating ODN sequence selectivity. Conclusions: These data demonstrate that in salt-resistant SD rats fed a chronic high NaCl intake, PVN Gaq protein pathways are endogenously suppressed to augment an essential counter-regulatory role of central (presumably PVN) Gaz protein signaling pathways, which inhibit salt-induced increases in AVP secretion, water retention and the development of hypertension. AHA 2250585, DK43337, HL071212, AHA 0855293E, P20 RR018766.

BC-O2. Aldose Reductase-Dependent Effects on Endothelial Dysfunction and Endothelial Progenitor Cells in Diet-Induced Obesity

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Laura Wheat, University of Louisville
Daniel J. Conklin, University of Louisville
Timothy E. O'Toole, University of Louisville
Aruni Bhatnagar, University of Louisville

Long-term diabetes is associated with an increase in cardiovascular disease risk. Long-term hyperglycemia in diabetes increases non-glycolytic consumption of glucose via the polyol pathway, which has been linked to the development of secondary diabetic complications. To examine the contribution of this pathway, wild-type (WT; C57BL/6) mice and mice deficient in aldose reductase (AR-null), an enzyme that catalyzes the first step of the pathway, were fed normal chow (NC, 13.5 % kcal fat) or a high-fat diet (HFD, 42% kcal fat) for 12 weeks. In comparison with WT mice (n=12), AR-null (n=12) mice gained significantly more weight (+14.9±0.9 g vs. +20.8±1.1 g; P<0.05) and displayed greater increase in plasma cholesterol (+133±213 mg/dl vs. +194±17 mg/dl; P<0.05) and insulin (+0.40±0.11 vs. +0.57±0.07 ng/ml; P<0.05). Insulin (100 nM) exposure increased Akt phosphorylation in isolated aorta of WT NC mice but not of AR-null mice. Aorta isolated from both HFD WT and AR-null mice were insulin-insensitive. High-fat feeding decreased acetylcholine-induced relaxation of aorta measured ex vivo in AR-null mice by 25±4 % (P<0.05, n=12) but not in WT mice. High fat feeding decreased the levels of Sca+/Flk+ endothelial progenitor cells (EPCs) in the peripheral blood (per 50,000 events: 197±79 vs. 103±19; P<0.05) yet increased EPCs in spleen (812±456 vs. 2088±1173; P<0.05) and bone marrow (432±269 vs. 1031±658; P<0.05) in WT mice. These changes were absent in AR-null mice. Collectively, these data suggest that AR-dependent metabolism of glucose or lipid peroxidation products during pre-diabetic conditions plays a significant role in the maintenance of endothelial health perhaps due to impaired insulin signaling and effects on circulating EPCs.

BC-O3. Ccm1p, a pentatricopeptide repeat protein, is essential to accumulate mitochondrial 15 S rRNA in *Saccharomyces cerevisiae*

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Kerry R. Belton, Alcorn State University
J. Ignacio Moreno, Alcorn State University

Mitochondrial dysfunction has been reported in aging and in a number of human diseases and disorders, such as cancer, diabetes, heart failure, and neurodegeneration. Specifically, alcoholic liver disease affects the structural and functional integrity of the mitochondrial ribosomes (mitoribosomes) and therefore, severely compromises protein synthesis in this organelle. Still, the molecular bases for mitochondrial deterioration are poorly understood. In human and yeast, a newly described class of proteins, which contain pentatricopeptide repeat (PPR) domains, are exclusively located in mitochondria and are essential for efficient transcription and translation. The baker's yeast, *Saccharomyces cerevisiae* is the ideal model organism for mitochondrial studies because it can live without this

organelle. Our laboratory is elucidating the effects of Ccm1p, a yeast PPR protein, on mitochondrial RNA metabolism. Ccm1p reportedly interacts with Mrp5p, a protein of the mitochondrial ribosomal small subunit. We have recently shown that Ccm1p was essential for the maturation of specific mitochondrial pre-mRNAs in a strain whose mitochondrial DNA contains introns [Moreno et al., *Current Genetics* 55:475-484 (2009)]. However, Ccm1p was also required for mitochondrial functionality in an intronless strain, since in the absence of this protein, the corresponding null mutants were unable to grow on non-fermentable substrates. This growth deficiency indicated lack of mitochondrial functionality. In the null mutant, the mitochondrial genome copy number was slightly decreased in comparison with that of the wild type. However, RNA hybridization analyses showed that the mutants specifically failed to accumulate mitochondrial ribosomal RNA of the small subunit (15S rRNA) over time. Protein expression experiments revealed that Ccm1p levels closely correlated with the amount of 15S rRNA. No other mitochondrial RNA, including the ribosomal RNA of the large subunit (21S rRNA) appeared to be affected by the absence of Ccm1p. Interestingly, even though the PPR domains are required for both activities, these reside in different amino acids. Based on these findings, we postulate that Ccm1p is a dual-functioning protein involved in mitochondrial pre-mRNA maturation and stability of ribosomal RNA. (This work was supported by PHS Grants 5P20RR16476 from the National Center for Research Resources and 1S3GM087169 from the National Institute of General Medical Sciences).

BC-O4. Cross-Talk Among O-GlcNAc Modification and Phosphorylation of Insulin Receptor Substrates

Mary Berkaw, Medical University of South Carolina
 Maria Buse, Medical University of South Carolina
 Lauren Ball, Medical University of South Carolina

The insulin receptor substrate (IRS) proteins are critical to the metabolic and mitogenic effects elicited by insulin and insulin-like growth factor (IGF-1). Upon hormone stimulation, IRS-1 and IRS-2 are phosphorylated by insulin/IGF-1 receptors which create binding sites for downstream interacting partners Grb2 and PI3K which signal to the MAPK and Akt pathways, respectively. These protein interactions and the duration of the signal are modulated by S/T kinases and possibly by O-GlcNAc glycosylation of S/T. Under conditions of nutrient excess and insulin resistance, IRS-1/2 are O-GlcNAc modified. The purpose of this study is to identify the sites of O-GlcNAc modification and putative cross-talk among PTMs on these highly phosphorylated adaptor proteins. IRS proteins were expressed in HEK293 cells in the presence of the O-GlcNAcase inhibitor, PUGNAC (50 .M). The proteins were enriched by affinity chromatography, gel purified, reduced and alkylated, and proteolytically digested. Peptides were analyzed by LC-MS/MS with an LTQ ion-trap MS equipped with ETD (Thermo). LC-MS/MS of IRS-1 yielded 80% sequence coverage of the 131 kDa protein and revealed multiple O-GlcNAc modified peptides which were detected in a phosphorylated and unphosphorylated state. Using a site-specific anti-O-GlcNAc antibody, the extent of O-GlcNAc modification of IRS-1 at S1011 increased in osteoblasts and hepatocytes treated with an O-GlcNAcase inhibitor suggesting that this modification dynamically cycles under normal conditions of cells growth. O-GlcNAc modification of IRS-2 occurred at known sites of S/T phosphorylation and thus may serve to mutually exclude phosphorylation at these sites. Both proteins were O-GlcNAc modified in close proximity to SH2 domain binding motifs and within domains known to interact with the insulin/IGF-1 receptors. Analyses are ongoing to elucidate the temporal dynamics of these modifications and their impact on insulin and

IGF-1 signaling in liver and bone with the goal of understanding how glucose-induced O-GlcNAc modification may contribute to the complications associated with diabetes.

BC-O5. The Folin-Ciocalteu Assay as a Valid Means of Assessing Antioxidant Activity of Compounds of Biomedical Interest

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Richard B. Walker, University of Arkansas at Pine Bluff
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Background and Objective: In our laboratory we are synthesizing potentially nephro-protective metal complexes. We currently employ several antioxidant assays in the initial screening of our compounds. The Folin-Ciocalteu (FC) assay has been used for over eighty years to measure proteins and phenols. However, recent evidence suggests that it functions as a general antioxidant assay. Our goal was to test this hypothesis and determine whether or not this assay is useful for measuring the antioxidant capacity of our metal complexes.

Methods: Over eighty compounds of several different classes were tested for reactivity towards the FC assay. These included the copper (II) and zinc (II) complexes of salicylate and aminothiols which have been synthesized in our laboratory. Reactivity of each compound was compared to that of gallic acid, which was used as the standard. Kinetic profiles were also run for compounds showing significant activity.

Results: All phenols and thiol derivatives tested were reactive towards the reagent. The inorganic ions sulfite, iodide, iron (II) and manganese (II) were also active. The trioses glyceraldehydes and dihydroxyacetone, the vitamin derivatives ascorbic acid, retinoic acid, Trolox, pyridoxine and thiamine, and the nucleotide base guanine displayed significant activity. Except for the thiazolidine derivative 2-propylthiazolidine-4-carboxylic acid (PTCA) which demonstrated slow kinetics, all other compounds tested displayed fast kinetics with half-lives less than one minute. All of the compounds synthesized in our laboratory were reactive towards the assay. Complexation with Cu(II) and Zn(II) had little effect on the reactivity of ligands towards the FC reagent.

Conclusions and Discussion: Our study showed that the FC assay is not specific for phenols and is reactive towards most compounds having reducing capability. Since it is a very simple method which gives very reproducible results, it is useful as a general measurement of antioxidant capacity. We will add this assay to the other antioxidant assays currently used in our laboratory. NIH Grant Number P20 RR-16460 from the IDeA Networks of Biomedical Research Excellence (INBRE) Program of the National Center for Research Resources. The Arkansas Space Grant Consortium also contributed funding towards this project.

BC-O6. Mass spectrometry for screening sphingolipid profile in human plasma: identification of irregular profile in disease

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 Jason S. Pierce, Medical University of South Carolina
 Farzan Soodavar, Medical University of South Carolina
 Barbara Rembiesa, Medical University of South Carolina
 Yusuf A. Hannun, Medical University of South Carolina
 Jacek Bielawski, Medical University of South Carolina
 Alicja Bielawska, Medical University of South Carolina

Sphingolipids in the blood constitute part of the circulating lipoprotein particles (HDL, LDL, and VLDL), carried by serum albumin, and present in blood cells and platelets. Several clinical studies have begun to address certain sphingolipid species as biomarker for disease. In these studies, the standardization and validation process of the sphingolipid analysis is still missing. The objective of this study was to develop and validate a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for determination of "normal" sphingolipid profile in human serum/plasma, and to identify certain changes in the profile as a biomarker for disease. Blood samples were collected from pre-screened healthy males (n=5) and non-pregnant females (n=5), under fasting and non-fasting conditions. Plasma was collected using EDTA, citrate, or heparin as anticoagulant. Sphingolipids analyzed included sphingoid bases sphingosine (Sph) and dihydrosphingosine (dhSph) and their 1-phosphates (S1P and dhS1P), and molecular species (Cn-) of ceramide (Cer), sphingomyelin (SM), glucosylceramide (GluCer), lactosylceramide (LacCer) and ceramide 1 phosphate (Cer1-P). The data show that SM was the dominant serum/plasma sphingolipid equally in both males and females, with C16-SM the highest of the SM species (64 μM) and most responsive to fasting conditions (100 μM). C24-Cer was the dominant ceramide (4.0 μM), with no effect of gender or fasting state on its levels. However, levels of medium chain ceramides (C16, C18, and C20) were decreased in response to fasting. As expected, serum S1P levels were significantly higher than plasma levels (0.68 μM vs. 0.32 μM , respectively). Fasting plasma levels of S1P showed a tendency of being higher in males. Among the Cer1-P species, C26-Cer1-P was higher under fasting conditions (> 2 fold). C16-LacCer was the dominant lactosylceramide (10.0 μM). Plasma collected in EDTA showed less variability in sphingolipids levels compared to heparin and citrate. However, differences in GluCer levels under fasting conditions were significant only when heparin was used as anticoagulant. Development and validation of a method to determine levels of circulating bioactive sphingolipids in humans reported in this study would provide a useful tool for clinical laboratory testing of sphingolipid profile in blood, and could be analogous in importance to blood cholesterol and triglyceride levels.

BC-P1. Cytotoxicity and Mitochondrial Effects of Di-Ethylhexyl and Mono-Ethylhexyl Phthalates

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 Beatriz Zayas, Universidad Metropolitana

The main objective of this study is to determine the toxicity of the phthalates Di-ethylhexyl (DEHP) and its principal metabolite, Monoethylhexyl (MEHP) on a human lymphocyte cell line, TK-6. Phthalates are a family of compounds used widely in the manufacturing industry and in the production of plastics. Human exposure to these

compounds can be through personal and consumer items like health care and beauty products. Phthalates are considered estrogen disrupters also effects on the reproductive and respiratory systems have been reported by the scientific community. Studies on the identification of biomarkers of exposure to phthalates, however, are limited. The analysis of the effects of phthalates on normal lymphocytes will allow the development of biomarkers of exposure to phthalates. TK-6 lymphocytes cells were cultured on 25cm² flasks on modified RPMI culture media with 10% FBS, and incubated at 37°C and 5% of CO₂. TK-6 cells were exposed to DEHP or MEHP at doses ranging from 5M, to 250M for 48 and 72 hours, the cytotoxicity was assessed by the Trypan Blue exclusion protocol. For apoptosis related analysis cells are being treated with the respective IC50 concentrations for 48 hours. Preliminary ROS Assays have been performed for qualitative and quantitative analysis. But also several tests related like the mitochondrial membrane permeability are in process to be determined by fluorescent analysis with MITO PT with the use of Valinomycin (permeate mitochondrial membrane) and Staurosporine, an apoptotic agent, as positives controls. The preliminary cytotoxicity analysis with DEHP and MEHP indicate IC50s of 20M and 36M respectively.

BC-P2. Cannabis: Old Plant, New Constituents, and Potential Therapeutic Agents

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Cannabis is very complex in its chemistry due to the vast number of its constituents and their possible interaction with one another. These compounds represent almost all of the chemical classes, e.g. mono- and sesquiterpenes, sugars, hydrocarbons, steroids, flavonoids, nitrogenous compounds and amino acids, among others. The best-known and the most specific class of cannabis constituents is the C₂₁ terpenophenolics, the cannabinoids, with (-)-9-trans-(6aR,10aR)-tetrahydrocannabinol (.9-THC) being the most psychologically active constituent. The availability of high potency marijuana on the illicit market with unprecedented .9-THC concentrations (>20% by dry weight), has renewed our interest in the discovery of new constituents from *C. sativa* L. As a part of our program aimed at the discovery of psychoactive constituents from *C. sativa* L., we herein report the binding affinities of minor isolated constituents to CB₁ and CB₂ receptors as well as the functional activity of some of them. Fifty two isolated compounds were evaluated in CB₁ and CB₂ receptor binding assays in Sf9 membrane preparations obtained from Perkin Elmer using [³H]CP55,940 as the radioligand. The CB₁ receptor binding assays showed that two compounds have significant binding affinities (0.8 and 5.5 nM, respectively), much greater than that of .9-THC (100 and 20 fold, respectively). Seven compounds have affinities (46.2-141.6 nM), comparable with that of .9-THC. On the other hand, four compounds showed lower affinities for CB₁ receptors (224.1-552.9 nM) and other four compounds were essentially inactive (725-2,263 nM). Five compounds showed significant binding affinities (5.9–116.0 nM) for CB₂ receptors. Two of them were very selective since they showed no affinities for

CB1 receptors. One compound showed the highest binding affinity for both CB1 and CB2 receptors, exceeding the synthetic positive control, CP55,940.

BC-P3. Study of the underlying factors that shape enzyme properties in organic solvents

Gabriel L. Barletta, University of Puerto Rico - Humacao

Enzyme catalysis in organic solvents has proven to be a valuable tool for the organic chemist. This is due to the fact that most enzymes remain catalytically active and highly enantioselective in this non-aqueous media. However, there are still mayor obstacles to overcome before their potential can be fully exploited. Some of the most challenging hurdles to overcome are the high influence of organic solvents on enzymatic activity, enantioselectivity and stability. For example, it has been well documented that an enzyme's activity and enantioselectivity are highly dependent on the physicochemical properties of the organic solvent used as the reaction medium. In addition, enzymatic activity diminishes exponentially during prolonged exposure to this medium. During the last four years our laboratory has studied the factors that govern the mechanism of enzyme catalysis in organic solvents, paying particular attention to enzyme structure, dynamics and active-site polarity. Results addressing these issues, obtained with the serine protease subtilisin Carlsberg will be presented. Among these are EPR and H/D exchange studies (completed using NMR and FTIR techniques) to determine the flexibility of the enzyme in several solvents; FTIR studies to determine the enzyme's secondary structure and a fluorescence spectroscopy study to determine the effect of solvents on the enzyme's active-site polarity.

BC-P4. The Transformation of Rubus and its Application to the Study of Plant Secondary Metabolites in Plant and Animal Cells

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Transformation is the genetic alteration of a cell resulting from the uptake and expression of foreign genetic material (DNA). The ability to perform transformation is a technique that has yet to be accomplished in the blackberry. To this end, we used Agrobacterium-mediated transformation techniques to transform the blackberry (*Rubus* spp) in tissue culture. A vector containing a GFP reporter gene, was used to produce transgenic tissue. Results thus far have revealed the successful transformation of blackberry callus and studies are ongoing. We intend to use this system to aid in the study of secondary metabolites in *Rubus*. Plant secondary metabolites provide many leads for new therapeutics that are currently on the market, however, their mechanism of action is often not understood in either plants or animals. We hypothesize that phenolics (a class of secondary metabolites) represent a class of plant hormones that activate developmentally-regulated signal transduction pathways and are also able to activate mammalian signaling pathways by mimicking small ligands.

BC-P5. Increase MAP in the IUGR male is associated with increases in oxidative stress and a difference in oxidant status mechanisms

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Norma Ojeda, University of Mississippi Medical Center
Thomas B. Royals, University of Mississippi Medical Center
Danielle Trocquet, Tougaloo College
Barbara Alexander, University of Mississippi Medical Center

Placental insufficiency in the rat leads to low birth weight with development of hypertension in male intrauterine growth restriction (IUGR) offspring; however, female IUGR are normotensive indicating sex differences in response to fetal insult. We previously reported that renal superoxide production is significantly elevated in male IUGR versus male Control, an increase abolished by treatment with the superoxide mimetic, TEMPOL (1 mMol/L for 2 weeks). Furthermore, chronic tempol also abolishes hypertension in male IUGR suggesting oxidative stress contributes to hypertension in male IUGR. In this study we tested the hypothesis that oxidant status is maintained through different mechanisms in male and female IUGR; thus, leading to sex differences in IUGR blood pressure. We examined the main antioxidant enzymes including CuZn superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and the mitochondrial enzyme, manganese superoxide dismutase (MnSOD). We observed protein expression of the anti-oxidant enzymes, GPX and catalase were significantly decreased in normotensive female IUGR as compared to hypertensive male IUGR, a decrease of 19% and 63%, respectively. The protein expression of MnSOD was significantly increased in the IUGR female as compared to the IUGR male. The protein expression of Cu/Zn SOD was not significantly different. After TEMPOL treatment the expression of MnSOD and Cu/Zn SOD were approximately the same in both female IUGR and male IUGR, suggesting that the superoxide mimetic reduces the expression of the two endogenous SOD enzymes. Thus, these studies indicate that sex differences in IUGR blood pressure may involve an imbalance of selected anti-oxidant enzymes. This research is supported by NIH-NCRR USM-GR036563-05-8S1, NIH-MHHD-1P20MD002725-01.

BC-P6. Measurement of uptake, accumulation and distribution of absorbed nanoparticles

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TJ Eskridge, Arkansas State University
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Alexandru Biris, University of Arkansas at Little Rock
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We have characterized the uptake of semiconductor nanocrystals (QDs) from water suspensions by a daphnid (*Ceriodaphnia dubia*) and fish (the fathead minnow, *Pimephales promelas*) and transfer of absorbed QDs between trophic levels in a simple laboratory based food chain. We have also used Raman spectroscopy to detect and map the distribution of single walled carbon nanotubes (SWCNTs) inhaled by mice. Results of these experiments have shown that:

1) *Daphnia* showed significant increases in fluorescence after exposures to pM QD concentrations. Increases in fluorescence (detected by fluorescence microscopy) were largely confined to the digestive tract, and were exposure and dose dependent.

2) After feeding upon algae that had absorbed significant amounts of QDs (as detected by fluorescence microscopy) increases in fluorescence were detected in *daphnia*. This result suggests that QDs can be transferred between trophic levels and may bioaccumulate as they pass through a food web. Experiments are underway to determine concentrations of accumulated QDs.

3) Fathead minnows exposed to nM concentrations of QDs showed significant increases in fluorescence in an exposure and dose-dependent manner. The concentration of absorbed QDs was measured using a fluorometer. This showed that animals exposed to 2 nM QDs for 5 hr had QD concentrations exceeding 100nM in their gut. After a 24 hr exposure, gut concentrations were ~200 nM. Experiments are underway to determine clearance rate of absorbed QDs.

4) Raman spectroscopy was used to examine slices of lung tissue from mice that had inhaled a water aerosol containing 20 ppb SWCNTs. This showed that SWCNTs were able to penetrate deep into the lungs of exposed animals. Mapping of SWCNT distribution in lung slices showed that although CNTs accumulated in small bronchioles, they were also found throughout the tissue. These results suggest that inhalation of CNTs results in their accumulation within and distribution throughout the lungs. Additional experiments are underway to determine if, after entering the lungs, inhaled SWCNTs enter the blood stream and partition into other tissues. These results suggest that these nanoparticles are enter organisms under realistic exposure conditions, can be accumulated against significant concentration gradients and bioaccumulate in food webs. Supported by: NIH NCRR grant P20 INBRE RR-16460, the UALR Center for Nanotechnology and the: Arkansas Biosciences Institute.

BC-P7. Analogue Synthesis of the Antibiotic Cytosporone E

Erin M. Cartwright, College of Charleston
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The antibiotic Cytosporone E is being used as a template to synthesize derivatives that are potentially more potent than the parent compound. Specifically, there are three alterations of focus for these derivatives: the selective deletion of oxygen atoms in order to find the pharmacological core of the molecule; substitution of the seven carbon side chain to alter functionality; and the incorporation of nitrogen to provide a new site for side chain addition. These derivatives of the parent antibiotic will be tested on both gram-positive and gram-negative bacteria in order to determine the relationship between cytosporone E's structure and its biological activity. This structure activity relationship study will provide information for developing more effective analogues of the parent antibiotic, which are needed with the ever-increasing number of antibiotic-resistant bacteria.

Bioinformatics

BI-O1. Mapping of International Protein Identifier to Affymetrix probe-set to Facilitate Biomarker Identification in Multiple Myeloma

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Affymetrix microarray is widely used in the field of genomics. Similarly, the International Protein Index (IPI) at European Bioinformatics Institute is a standard protein database, commonly used in proteomics studies. Affymetrix provides mapping annotations to link some, but not all Affymetrix probe-set identifiers to the protein identifiers in databases like SwissProt and RefSeq protein. Likewise, IPI provides mapping annotations to link some, but not all IPIs to the available gene identifiers in databases like UniGene, Entrez Gene. Moreover, since most of the systems biology tools including GO annotation, are optimized for genomics data, most proteomics data are essentially converted to genomics-compatible dataset. Hence, critical information is lost in the conversion process due to incomplete annotation resulting in incorrect analysis. Thus, a comprehensive mapping between proteomics and genomics databases is required to enable correlation of these two expression profiles. Therefore, we created mapping tables to link each of the IPI to a corresponding Affymetrix identifier. Our mapping tables provide either direct or indirect mappings. Direct mappings are obtained by parsing the annotation flat files provided by IPI and Affymetrix, and establishing a link using all possible common database identifiers. Indirect ones are based on alignment procedures using Basic Local Alignment Search Tool (BLAST). Thus, 83.3% of the human protein IPI dataset was connected via direct mapping to corresponding Affymetrix identifiers, and the remaining 16.7% were subjected to BLAST analysis. Evaluation of the mapping obtained by our annotation tables, to that obtained by using open source mapping tools is in progress. Furthermore, proteomic data (IPI identifiers) of 100 multiple myeloma (MM) baseline samples will be converted to Affymetrix identifiers and compared to already available genomic data generated via Affymetrix platform. We can get a full representation of the correlation between proteomics and genomics data by using our mapping tables, since it represents a more complete mapping, obtained by using both direct, and indirect i.e. alignment based mapping links. The proteomics-genomics correlation studies might further aid in diagnosis and/or prognosis of MM, by subsequently finding a biomarker pattern, consistent across both proteomics and genomics profiles. These unique biomarker patterns, corresponding to high risk and low risk MM groups (Blood. 2007 Mar 15; 109(6):2276-84., Shaughnessy JD Jr. et al), can have significant implications on the individual course of treatment.

BI-02. Genome GC content in bacteria is related to the presence or absence of genes involved in base excision DNA repair

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GC content is a key characteristic of genomes, yet the cause of differences in genome GC contents awaits a satisfactory mechanistic explanation. In theory, the presence of a DNA repair pathway in a genome which is biased in the types of mutations it corrects, has the potential to alter the underlying GC content by preferentially correcting certain mutations. Here, we show that bacterial genome GC content is related to the presence or absence of genes involved in base excision DNA repair. Base excision repair is a biased DNA repair pathway; the products of the *mutM* and *mutY* base excision repair genes both correct GC->AT mutations. In contrast, the mismatch repair pathway is unbiased, in that it shows no preference for the types of mutations corrected. The presence of the *mutM* and *mutY* genes are correlated with genome GC content, consistent with their involvement in biased DNA repair. In contrast, the presence of the mismatch repair *mutS* and *mutL* genes are not, consistent with their involvement in unbiased DNA repair. In addition, it is shown that bacteria with smaller proteome sizes are more likely to lack DNA repair genes. These results are predicted by the Proteomic Constraint theory, which proposes that the selective pressure to maintain and evolve DNA repair is reduced in genomes/proteomes that undergo a reduction in size. In summary, the results imply that a proportion of genome GC content in eubacterial genomes has arisen non-adaptively as a result of the presence or absence of genes involved in base excision repair, which in turn is influenced by proteome size.

BI-03. Sequencing and Annotation of the Bacteriophage HK239 Genome

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Temperate bacteriophage can replicate to generate new phage or they can adopt a lysogenic lifestyle in which they incorporate their genome into the bacterial chromosome, creating a prophage. Most prophage genes are not expressed. However, those that are have diverse functions ranging from virulence to exclusion. Bacteriophage HK239 is of particular interest since it is capable of excluding a wide range of phage including λ , P2, f80, T4rII mutants, P1vir, and HK022. We have shown that the exclusion of HK022 is due to the activity of a f80 *cor* gene homolog carried by the phage. To learn more about HK239 and to identify additional exclusion genes, the genome was sequenced. A library was generated by shearing HK239 genomic DNA and then ligating the fragments into a pSMART vector. Random clones were sequenced and the fragments were assembled based on overlapping sequence. Gaps were closed by sequencing directly from HK239 DNA. The assembly was verified by restriction analysis and additional sequencing as needed. Open reading frames have been identified using Viral Genome Organizer and Genemark and the remaining annotation is nearly complete. No homologs to genes that express known exclusion functions have been identified. However, three novel genes that are of interest will be characterized.

BI-P1. Molecular Investigation of Prickle Development Genes: A Model for Cell Communication

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Prickles are outgrowths of epidermal and sometimes cortical plant tissue that develop from a signaling cascade initiated by the head of glandular trichomes. These signals result in the division and growth of epidermal and underlying cortical cells. Due to the simplicity of their structure, prickle development is an ideal model to investigate how cells communicate to control growth, proliferation, and morphological differentiation. Understanding these modes of cellular communication could be a significant factor in all developmental pathways including mammals. We are currently analyzing gene candidates and their function in prickle development to better understand the role of trichomes in prickle development. To this end, we are currently identifying orthologous ESTs (expressed sequence tags) from the trichome developmental pathway of *Arabidopsis* and other model species using a degenerate primer method. We have thus far identified several genes from raspberry and blackberry, our prickle development model plant. Using these orthologous EST sequences we are carrying out functional analysis using in situ hybridization. These data will provide some insight as to the potential signaling pathways involved in prickle development and may provide some clue to similar growth and developmental mechanisms in mammalian cells.

BI-P2. mRNA Expression analysis using microarrays and sequencing

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We analyzed mRNA expression using two widely-used genomics technologies: microarrays and sequencing. We correlated the mRNA expression levels obtained from both technologies. In this analysis, high-quality mRNA samples from brain and cancer were used which had also been used for the FDA MAQC project. Microarrays probes were then mapped against NCBI reference sequences. Perfectly matched probes were considered for further analysis; this involved extending the window size of the probe search. Probes which are mapped against the non-coding region were not considered for this analysis. Fifty nucleotide probes were extended on both sides in order to obtain a wider picture of the transcription region of the genome. Expression results obtained from microarrays were normalized and significantly-expressed and non-expressed genes were considered for statistical analysis. Differentially-expressed genes in brain and cancer cells were identified. Overall unexpressed genes were also identified in this experiment. Reads from the sequencing were preprocessed and mapped against the same reference. Differentially-expressed genes from both technologies were compared and analyzed. Pros and cons related to both technologies were encountered and variability across the technologies identified.

BI-P3. Anthropomorphic Question Answering System for the -Omic Era

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In clinical and biomedical settings researchers use specialized search engines to acquire answers to technical questions or to quickly get verification of experimental results. The outcome of such queries result in the reading and scanning of multiple Web pages and documents. Question answering (QA) is a specialized type of information retrieval with the aim of returning precise short answers to queries posed as natural language questions. We propose a QA system that creates a dialog with the user that mimics human interaction to answer biological questions. The QA system is designed to be modular to allow for easy modification of core components. The system's architecture can be divided into four subsystems: knowledge base, question analyzer, answer identifier, and answer presenter. Multiple software agents create the knowledge base and find possible answers to questions, from which the most relevant will be presented to the user. A dialog established with the user can obtain feedback to refine the query. Answers will be automatically marked-up and linked to semantically relevant content in other databases. The additional information will be presented in a popup window that appears when a marked term is clicked. The proposed system addresses two current requirement gaps in question answering, namely, incorporating multimedia information and an ability to interact with the user. There is a lack of systems that allow the user to choose his or her environment, establish context, have the system take that information into account, and automatically return the appropriate answer. The QA system is intended to return short answers to biological questions which will eliminate the need to read entire document. The QA system is expected to decrease the amount of time required to find answers when compared to traditional information retrieval methods (e.g., PubMed).

Cancer Research

CR-O1. Novel Bovine Coronavirus-based Therapeutics for Human Colorectal Cancer

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One of the newest technologies being developed to fight cancer is oncolytic virotherapy (the destruction of tumors using viruses). This revolutionary approach is undergoing testing in various pre-clinical and clinical models. Colorectal cancer [CRC] is the second most common solid internal malignancy with an estimated 148,810 new cases expected to be diagnosed in the U.S. in 2009. Prevention of this malignancy at an early stage of development is considered the key to reducing CRC mortality. Current preventative strategies rely on visual screening, a technique which misses small malignant growths. The main hypothesis of our current investigations is that bovine coronavirus (BCV), which is known to selectively infect human colon cancer cells in cell culture can be utilized as a novel preventative and therapeutic treatment for human colorectal

cancer. BCV has the unusual property of replicating to high titers in human colorectal tumor cells (HRT), while it is generally not infectious for most human cells tested in tissue culture. We have established an in vivo model for CRC progression in nude mice. Initial experiments have showed that BCV treatment of these human tumors prevents or reduces growth compared with vehicle-injected controls. BCV replicates efficiently in different tumor cell lines including human rectal tumors cells (HRT) producing pronounced cytopathic effects characterized by extensive virus-induced cell fusion. The aims of our INBRE-funded project are: 1) to test BCV infection of human colon cancer cells in culture to determine appropriate viral titers for efficient oncolytic therapy; 2) To test BCV infection of human colon cancer cell in a mouse model to determine the efficacy of using BCV to prevent and/or destroy colon tumors formed in nude mice. It is envisioned that specific BCV strains that are utilized in this study may be used in the future to treat human colorectal cancer via non-invasive procedures. In this regard, the ability of the virus to survive passage through the gastrointestinal tract may enable the use of oral-based treatment for this type of cancer.

CR-O2. Racial differences in prevalence of HPV associated head and neck carcinoma: is chronic inflammation a cofactor?

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South Carolina exceeds the US incidence rate for head and neck squamous cell carcinoma (HNSCC), ranking 3rd in the nation in mortality. African Americans (AA) have a higher incidence of HNSCC, and suffer a mortality rate that is twice that observed in European Americans (EA). Recently, it was found that ~25% of all HNSCC, and 60% of oropharyngeal cases, are associated with high-risk human papillomavirus (HPV) type-16. HPV-positive HNSCC forms a clinically and molecularly distinct subset of the disease, which has increased in incidence over 20 years. HPV-positive disease carries an improved prognosis, with significantly improved overall and disease-specific survival. We hypothesized that the difference in HPV-positive disease survival might be a factor in the cancer disparity, and initiated a pilot study, measuring HPV-16 levels in HNSCC specimens from 74 patients (51 EA and 23 AA) treated at the Hollings Cancer Center. Preliminary evidence indicated that the AA patients had a much lower prevalence of HPV-positive HNSCC (3/23, 13.0%), compared to stage-matched EA patients (27/51, 52.9%). These results were highly significant ($p = 0.002$; 2-tailed Fisher's exact), and suggest that AA patients appear to be particularly susceptible to more aggressive HPV-negative tumors. Traditional risk factors for HPV-negative disease include heavy tobacco and alcohol use, and chronic inflammation resulting from periodontal disease. We thus examined the differences of these factors between racial groups. The susceptibility to HPV-negative HNSCC could not be explained by smoking and alcohol history, which did not differ between racial groups, suggesting a role for the other main etiologic risk factor periodontal disease. We thus measured tooth loss as an indication of chronic periodontal disease between racial groups, finding that AA patients had significantly

more tooth loss than did EA patients. In summary, significant health disparities exist for outcomes of AA HNSCC patients compared to EA HNSCC patients, with AA patients having worse survival and disease recurrence. We hypothesize that the observed survival differences reflect differences in prevalence of HPV-positive HNSCC, which has a better prognosis than HPV-negative disease. Our data suggest that AA patients have higher rates of periodontal disease, a risk factor for HPV-negative HNSCC, possibly increasing AA susceptibility to this malignancy.

CR-O3. Autocrine Interleukin-6 De-Sensitizes LNCaP Cells to Endocrine/Paracrine Interleukin-6 Signal

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Introduction and Objectives: In LNCaP cells that secrete IL-6 (autocrine) either through IL-6 cDNA transfection (Clin Cancer Res, 2003, 9:370-6) or induced by long-term treatment with exogenous IL-6 (Clin Cancer Res, 2001, 7:2941-8; Prostate, 2007, 67:764-773), IL-6 promotes cell growth and protects LNCaP cells from undergoing apoptosis induced by androgen deprivation therapy. In contrast, LNCaP cells treated with exogenous IL-6 (mimicking endocrine/paracrine effect) undergo cell growth arrest and neuroendocrine differentiation (NED). The objective of this study was to investigate the differences between autocrine and endocrine/paracrine IL-6 signaling pathways in LNCaP cells. **Methods:** LN-S17 (LNCaP cells overexpressing IL-6) and control cell line LN-C3 (LNCaP cells not expressing IL-6) were compared in cell growth, NED marker expression, and intracellular signaling molecules by cell culture, Western blot, and real-time quantitative PCR. **Results:** LN-C3 cells underwent growth arrest and NED when they were treated with exogenous IL-6 or co-cultured with LN-S17 cells for 4 days, whereas LN-S17 cells continued to proliferate. LN-C3 cells expressed approximately the same amount of IL-6R, gp130, JAK1, JAK2, and Tyk2, compared to LN-S17 cells. JAK3 was not expressed in LNCaP cells. Phospho-JAK2 but not JAK1 or Tyk2 was induced in LN-C3 cells but not in LN-S17 cells upon exogenous IL-6 treatment. Phospho-STAT3 was minimal in LN-S17 cells but was dramatically induced in LN-C3 cells when both cell lines were treated with exogenous IL-6. LN-S17 cells constitutively expressed higher levels of cytokine-inducible SH2-containing protein (CIS) and suppressor of cytokine signaling 7 (SOCS7) than LN-C3 cells. The levels of SOCS1, SOCS2, SOCS3, SOCS4, SOCS5 and SOCS6 proteins were equal in both cell lines. **Conclusions:** Autocrine IL-6 de-sensitizes LNCaP cells to endocrine/paracrine IL-6 signal by increasing constitutive expression of CIS and SOCS7. **Acknowledgement:** DoD W81XWH-05-1-0567, NIH/NCRR 2P20 RR020152-06, and LCRC Fund.

CR-04. NRAGE interactions with TBX2 and ankyrin-G: a pathway for the control of anoikis by E-cadherin and EMT

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Detachment of epithelial cells from the extracellular matrix or attachment to an inappropriate matrix engages an apoptotic response known as anoikis, which prevents metastasis. Cellular sensitivity to anoikis is compromised during the oncogenic epithelial-to-mesenchymal transition (EMT) through mechanisms that are not yet understood. Herein, we report a pathway through which EMT confers anoikis-resistance. Neurotrophin Receptor-interacting MAGE (Melanoma Antigen) domain protein (NRAGE) was found to suppress anoikis. NRAGE was over-expressed in melanoma and carcinomas of the lung, breast and colon, and upregulated by experimental EMT induction. NRAGE interacted with the oncogenic transcriptional repressor protein T-box 2 (TBX2) and was a functional co-repressor protein for TBX2 (and TBX3), in that the repression of a TBX2 target gene, p14ARF, required the recruitment of NRAGE to the p14ARF promoter. TBX2 suppressed and p14ARF promoted anoikis. NRAGE also interacted with a cytoskeletal component of the E-cadherin complex that is frequently downregulated in human tumors, ankyrin-G. Ankyrin-G sequestered NRAGE in the cytoplasm. Induction of EMT down-regulated ankyrin-G, increased the nuclear localization of NRAGE and enhanced the repression of p14ARF, protecting cells against anoikis. These results indicate that NRAGE and TBX2 collaborate to suppress p14ARF expression and anoikis. E-cadherin and EMT affect ankyrin expression, modulating the co-repressor activity of NRAGE and controlling gene expression through TBX2, a novel pathway for the regulation of anoikis by EMT.

CR-05. Role for the Sphingosine kinase 1/Sphingosine-1-phosphate pathway in colon carcinogenesis

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Colorectal cancer remains the 2nd leading cause of cancer-related deaths in the US despite recent advances in screening and therapeutic technology. Recent studies suggest that bioactive sphingolipids are key in regulating the arachidonic acid cascade of inflammation, which is significant in colon cancer pathogenesis. Sphingolipid metabolites such as ceramide, sphingosine, and sphingosine-1-phosphate (S1P) are a new class of lipid messengers that regulate cell functions. Sphingosine kinase 1 (SphK1), phosphorylates sphingosine to form S1P, is a critical regulator of sphingolipid-mediated functions, as it not only produces the pro-growth, anti-apoptotic messenger S1P, but also

decreases pro-apoptotic ceramide and sphingosine. Our previous studies implicated the SphK1/S1P pathway in induction of the arachidonic acid cascade, a major inflammatory pathway involved in colon carcinogenesis (FASEB J., 20: 386-388, 2006). Then, we investigated whether the SphK1/S1P pathway is necessary for mediating carcinogenesis *in vivo*. We report that 89% (n=47) of human colon cancer samples stained positively for SphK1 whereas normal colon mucosa had negative or weak staining. Adenomas had higher SphK1 expression versus normal mucosa, and metastatic colon cancers had higher SphK1 expression than those without metastases. In an azoxymethane (AOM) murine model of colon cancer, SphK1 and S1P were significantly elevated in colon cancer tissues compared to normal mucosa. Moreover, blood levels of S1P were higher in mice with colon cancers than in those without cancers. Importantly, SphK1^{-/-} mice subjected to AOM had significantly less aberrant crypt foci (ACF) formation and significantly reduced colon cancer development. These results suggest that the SphK1/S1P pathway contributes to colon carcinogenesis and that inhibition of this pathway is a potential target for chemoprevention. In this paper, we discuss more details of the role of the SphK1/S1P pathway in colon carcinogenesis and the effects of SphK1 inhibition in cardiovascular risk.

CR-O6. Regulation of High Mobility Group A1 expression by the Wnt β -catenin signaling

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High Mobility Group A1 (HMGA1) proteins are diverse biological mediators with important roles in viral integration, modification of chromatin structure, neoplastic transformation, and metastatic progression. HMGA1 proteins are overexpressed in a variety of cancers. We previously showed that mice bearing the *hmga1a* transgene develop aggressive lymphoid malignancy resembling human T-cell acute lymphoblastic leukemia that is abrogated following treatment with inhibitors of the HMGA1 target, STAT3. Although this evidence supports a causal role for HMGA1 in malignant transformation, the molecular networks surrounding HMGA1-induced transformation are not clear. Gene expression profiling and proteomic studies suggest that the proteins acts by 1) activating inflammatory pathways, 2) inhibiting tumor suppressor function, and 3) driving the expression of cellular oncogenes. Consistent with these observations, HMGA1 is a novel target of *c-myc* and a transactivator of cyclooxygenase-2 COX-2 and cyclin D. Interestingly, these genes are all dysregulated in colon cancer resulting from impaired tumor suppressor function of adenomatous polyposis coli (APC). Mutations in the *Apc* gene are associated with the earliest stages of colon carcinogenesis. These mutations activate the Wnt signaling pathway, resulting in stabilization of β -catenin which then binds to T-cell factor-4 (Tcf-4) causing subsequent increases in target gene expression. Because HMGA1 expression is overexpressed in most cancers and has known interactions with several Wnt-responsive genes, we explored the involvement of HMGA1 in Wnt signaling. In *Apc*(Min/+) mice expressing truncated *Apc*, we observed 9-fold greater HMGA1 mRNA levels relative to mice bearing wild-type *Apc*. HMGA1 protein levels were also significantly elevated in *Apc* (Min/+) intestinal tumors. To assess the effects of wild type *Apc* recovery, we used HT-29 cells (human colorectal carcinoma cell line with truncated *Apc*) in which wild type *Apc* has been introduced under the control of a zinc inducible promoter. Induction of full length *Apc* caused down-regulation of HMGA1 at the translational level. Sequence analysis of the 5'-flanking sequence of

hmg1 identified three putative Tcf-4 binding elements and studies of the functional roles of these elements will further substantiate our findings. Our data implicates Wnt signal transduction in regulating HMGA1 and further expands the extensive regulatory network affected by Wnt/ β -catenin signaling.

CR-P1. IL-17A Stimulates Expression of Chemokines and Cytokine in Prostatic Epithelial Cell Lines

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Introduction and Objectives: T helper 17 (Th17) cells secrete interleukin-17A (IL-17A) and interleukin-17F. Th17 cells and IL-17A are increased in prostate tumors. The role of IL-17A in prostate cancer is not clear. This in-vitro study was to investigate the effects of IL-17A on prostatic epithelial cells. **Methods:** MTT assay, Western blot, and real-time quantitative RT-PCR analysis were used to measure the effects of IL-17A on immortalized normal human prostatic epithelial cell lines (RWPE-1 and pRNS-1-1), human high-grade prostatic intraepithelial neoplasia (PIN) cell line, human prostate cancer LNCaP cell line, and mouse prostate cancer TRAMP-C1 cell line. **Results:** Recombinant human IL-17A (20 ng/ml) did not affect cell growth rate in any of the prostatic cell lines studied. IL-17A did not significantly activate NF-kappaB or ERK signaling pathway in RWPE-1, pRNS-1-1, or PIN cells. IL-17A activated NF-kappaB and/or ERK signaling pathways in LNCaP and TRAMP-C1 cells. When IL-17RC (receptor of IL-17A) was overexpressed in PIN and LNCaP cells, activation of NF-kappaB or ERK signaling pathway by IL-17A was significantly enhanced. IL-17A modestly induced mRNA expression of chemokines (CXCL1 and CXCL2) in RWPE-1, pRNS-1-1, and PIN cells. IL-17A induced mRNA expression of chemokines (CXCL1, CXCL2, CCL2, CCL5, CCL7, and CCL20) and cytokine IL-6 in LNCaP cells. In mouse TRAMP-C1 cells, IL-17A induced mRNA expression of chemokines (CXCL1, CXCL2, CXCL5, CCL2, and CCL5) and cytokine IL-6. When IL-17RC was overexpressed in PIN and LNCaP cells, chemokine expression was significantly enhanced. **Conclusions:** IL-17A stimulates expression of chemokines and cytokine in prostatic epithelial cell lines, particularly in malignant epithelial cells. Although IL-17A has no effects on cell growth in-vitro, it is speculated that IL-17A-induced chemokines and cytokine may modulate the stroma-epithelial interaction in tumor microenvironment in-vivo. **Acknowledgement:** DoD W81XWH-05-1-0567, NIH/NCRR 2P20 RR020152-06, and LCRC Fund.

CR-P2. Understanding the Mechanism of the Anti-Antimitogenic Activity of Suramin

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Fibroblast growth factors potent mitogens that regulate key cellular processes such as differentiation, angiogenesis, and tumor growth and metastasis. Therefore, intensive research efforts are on to develop therapeutic principles against FGF-induced pathogenesis. FGFs exhibit their cell proliferation activity by binding to the extracellular D2 domain of their cell surface receptor. Suramin (carbonyl bis[imino-3,1-phenylenecarbonylimino(4-methyl-3,1 phenylene)carbonylimino]-bis(1,3,5-naphthalenetrisulfonic acid) hexasodium salt) has been previously shown to inhibit FGF-induced tumors. In this context, in the present study, we investigate the interaction of suramin with the extracellular D2 domain of the FGF receptor (FGFR). Results of the isothermal

titration calorimetry (ITC) experiments suggest that suramin binds to the D2 domain of FGFR with a reasonably high affinity ($K_d \sim 10^{-6}$ M). ITC experiments, carried out at various salt concentrations, showed that suramin-D2 domain interaction is stabilized by ionic and hydrophobic interactions. Size exclusion chromatography profiles of the D2 domain, obtained in the presence and absence of suramin, show that the drug binds to the receptor domain in a 1:1 stoichiometry. Equilibrium unfolding experiments monitored by far UV circular dichroism reveal that the D2 domain is significantly stabilized by suramin. ^1H - ^{15}N chemical shift perturbation data show that the suramin binding sites are mostly contributed by residues located at the N- and C-terminal ends of the D2 domain. Interestingly, some of the residues of D2 that bind to suramin are located at the FGF-D2 domain binding interface. Therefore, it appears that suramin inhibits the cell proliferation activity of FGF by preventing its interaction with FGFR. Several analogues of suramin are made and their interactions with FGF and D2 domain have been studied. The results of this study are expected to pave way for a rational design of drugs against FGF-induced tumors.

CR-P3. L1 element expression in human tissues and implications for cancer

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LINE-1 expression damages host DNA via insertions and endonuclease-dependent DNA double-strand breaks (DSBs) that are highly toxic and mutagenic. The predominant tissue of LINE-1 expression has been considered to be the germ line. We show that expression of the full-length and processed L1 transcripts is widespread in human somatic tissues. Northern blot analysis detects a significant variation in both L1 expression and L1 mRNA processing not only in normal human tissues but also in human cancer cell lines. Furthermore, RNA processing appears to be one of the major regulators of the amount of the full-length L1 mRNA detected in both human tissues and cancer cell lines. Many human tissues also produce translatable spliced transcript (SpORF2). An Alu retrotransposition assay demonstrates that this mRNA drives efficient Alu retrotransposition in cultured cells. COMET assays and 53BP1 foci staining show that the SpORF2 product can support functional ORF2 protein expression and can induce DNA damage in normal cells. Tests of the senescence-associated β -galactosidase expression suggest that expression of exogenous full-length L1 or the SpORF2 mRNA alone in human fibroblasts and adult stem cells triggers a senescence-like phenotype, which is one of the reported responses to DNA damage. In contrast to previous assumptions that L1 expression is germ-line specific, the increased spectrum of tissues exposed to L1-associated damage suggests a role for L1 as an endogenous mutagen in somatic tissues. These findings have potential consequences for the whole organism in the form of cancer and mammalian aging.

CR-P4. The interaction of anticancer copper complexes with DNA and human serum albumin

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A series of four copper compounds containing piperonal thiosemicarbazones have

been synthesized. The complexes show varied and novel structural motifs with the parent piperonal thiosemicarbazone forming a bimetallic compound and the phenyl substituted analog forming a mixed-tautomer complex - one thiosemicarbazone ligand is neutral and the other is anionic. The compounds are biological active showing promising anticancer activity against breast cancer cell lines (MCF-7 and MDA-MB-231) as well as colon cancer cell lines (HCT116 and HT29), with IC50 values ranging from 1.4 to 28 micromolar. Preliminary results suggest that the compounds do not have a strong interaction with DNA as they fail to cleave pBR322. Further investigations of the reaction on the compounds with DNA and human serum albumin using various spectroscopic techniques will be reported.

CR-P5. Development of Novel Anti-Cancer Agents

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Cancer is the leading disease-related killer in the US, and therefore there is tremendous need for effective new chemotherapeutic treatments. The ultimate goal of this research project is to discover and develop novel chemotherapeutic agents of more potency and less toxicity for treatment of cancer patients. Increasingly, researchers are attempting to optimize bioactive natural products in order to enhance their efficacy and minimize their toxicity. The primary goal of this research is to develop novel anti-cancer agents based on fusarochromanone (FC101a), a natural mycotoxin with potent anti-angiogenic and direct anti-tumor activity. Like most other bioactive natural compounds, the potency of FC101a is compromised in-vivo, suggesting rapid metabolism and/or poor bioavailability and distribution. Clearly, further development of FC101a as a chemotherapeutic drug requires that we complete its total synthesis, as well as the synthesis of a series of structural analogs that may show greater in-vivo potency. The creation of the parent FC101a and its analogs will allow for the analysis of quantitative structure/activity relationships (QSAR), using both in-vitro cellular and in-vivo animal models of tumorigenesis. Our research group has already synthesized a series of simplified FC101 analogs without the side chain, and we have conducted preliminary assays to determine the effect of these analogs on in-vitro cancer-cell viability and proliferation. In contrast to the parent compound, most of these new synthetic analogs that lack the side chain found in the natural product have increased cell viability and proliferation for all cell lines tested. This sharp contrast in activity indicates that the FC101 side chain is required for the anti-cancer and angiogenic activity of this molecule. Although the stimulation of proliferation may have profound applications (e.g., the stimulation of stem cell growth), we are focusing our current research efforts on the natural product itself as well as new side chain analogs that may find direct application in the treatment of cancer.

CR-P6. Development of a Novel Anticancer Agent Modeling Combretastatin A-4

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Combretastatin A-4 is a naturally occurring anti-tumor agent that binds specifically to the colchicine-binding site on tubulin, a key protein in the process of cell division. Both molecules (colchicine and combretastatin A-4) cause a decrease in elasticity, therefore preventing cell division. Although combretastatin A-4 is an effective anti-tumor agent, it is very cytotoxic, making it a less than ideal drug choice. The goal of this research project is to synthesize analogs of combretastatin A-4 that are less cytotoxic, while still maintaining potency against cancer. The synthesized analogs will differ from the structure of combretastatin A-4 by the addition of a lactone ring, which is attached to the double bond (making a phthalide derivative). This lactone ring is significant because, as a five-membered ring, it introduces ring-strain into the system as well straining the double bond, which alters the conformation of the molecule. Starting with a series of substituted 4-anisaldehydes, a sequence of reactions will be performed in order to obtain the derivatives.

CR-P7. Comparison of the Toxicity of BQs on Normal TK6 Lymphoblast vs A431 Tumor Cells

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 Wigberto Hernandez, Universidad Metropolitana
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 Beatriz Zayas, Universidad Metropolitana

This study evaluates the toxicity of four benzazolo [3,2-a] quinolinium drugs on normal T lymphocytes as surrogate of effect for other normal tissues in comparison with previously generated toxicity data on A431 tumor cells. The compounds under study, are nitro and amino containing heterocyclic compounds possessing a positive charge that could facilitate their interaction with cell organelles, especially mitochondria. Within the cytotoxic effects observed on A431, and under analysis on normal cells are mitochondrial damage, apoptosis and ROS release of BQs. Treated cells. Additional studies however are necessary to further understand the cell death pathway of cells exposed to BQs. Tested drugs on normal TK6 lymphoblast cell line were: NBQ38, & ABQ38. For determination of the IC₅₀ inhibition, TK6 cultures were exposed to BQs for 48 hours and cytotoxicity assessed by Trypan Blue exclusion. Preliminary results on TK6 indicated higher tolerance to the tested ABQ38 since an average IC₅₀ of 36 μ M were observed on A431 tumor cells. In the TK6 normal cells the IC₅₀ has not been observed yet even at 200 μ M. Higher concentrations need to be tested. The NBQ 38, however, demonstrated higher toxicity on the normal cell line with an IC₅₀ of 10 μ M in comparison with the IC₅₀ on the tumor cells of 40 μ M. Preliminary qualitative and quantitative results in A431 indicated abnormal generation of Reactive Oxygen Species (ROS). ROS generation was determined by fluorescence microscopy applying the reagent 2',7'- Dichlorofluorescein diacetate (20 μ M). Understanding how lymphocytes react to BQs' exposure as potential chemotherapeutic agents will provide information regarding the preferential selectivity of BQs toward tumor cells.

CR-P8. Understanding the Mechanism of Autoregulation of FGF Signaling

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Fibroblast growth factors (FGFs) are heparin binding proteins that help regulate key cellular processes such as wound healing and differentiation, cell proliferation, cell migration, morphogenesis, and angiogenesis. The FGF signaling is generated by the binding of the ligand (FGF) to the extracellular domain of the FGFR, this binding induces dimerization of FGFR, which is an essential step in FGF signaling. Fibroblast growth factor receptor (FGFR) extracellular domain consists of three Ig domains D1, D2, and D3. Between the Domains D1 and D2 is a short span of acidic residues called the “acid box.” The D1-D2 linker is thought to play a role in regulation of FGF interaction with FGFR. Many of the FGF binding sites can be found on the extracellular D2 domain of the receptor. It is believed that “acid box” can regulate FGF binding to FGFR. The “acid box” can mimic heparin like compounds and bind at the heparin binding sites located on the surface of the D2 region of FGFR. In the present study, we synthesized a twenty-eight amino acid box region peptide and studied its interaction with D2 domain of FGFR using various biophysical techniques including multidimensional NMR spectroscopy. Equilibrium unfolding experiment monitored steady state fluorescence, far-UV circular dichroism and proteolytic digestion experiments reveal that acid box binds to D2 domain very weakly. Two-dimensional nuclear magnetic resonance 1H-15N HSQC experiments show that the acid box binds to the FGF-1 and heparin binding sites in the N-terminal end of the D2 domain of FGFR. Our results clearly show that the acid box peptide binds to the ligand binding domain of the fibroblast growth factor receptor.

CR-P9. Copper Complexes as Scavengers of Reactive Oxygen Species and Cell Regeneration in TKPTS Cells.

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Background and Objective: The most common choice of treatment of cancer has been chemotherapy, though successful in killing cancer cells also kills the normal cells. Prior treatment of patients who are to undergo treatment with chemotherapy for their neoplastic disease with metal complexes may be beneficial to the patients. Copper complexes of salicylic acid were synthesized, characterized, and tested as catalytic antioxidants that scavenge a wide range of reactive oxygen species (ROS). The in vitro data collected in this study strongly indicate that these metal complexes satisfy many of the criteria for prevention and treatment of cisplatin-induced normal cell-toxicity, as they are active, stable, and nontoxic antioxidants. **Methods:** The antioxidant activities of copper complexes with dichlorosalicylic acid (DCS), diisopropylsalicylic acid (DIPS), ditertiarybutylsalicylic acid (DTBS) and dibromosalicylic acid (DBS) were evaluated using NBT assay. Toxicity of the complexes was tested in the TKPTS cells by measuring the lactate dehydrogenase (LDH) release. The caspase profiling was done using AMC Caspase Assay kit.

Results: All the copper complexes had very low value of percentage inhibition (IC50) indicating that they are good antioxidants. The results showed threshold levels of toxicity at higher concentrations TKPTS cells. The caspase profiling data clearly shows that these metal complexes are effective in lowering the caspases induced by cisplatin treatment. Conclusions and Discussion: These metal complexes may be useful agents in preventing cisplatin-induced cell toxicity as well as an anticancer agent capable of causing re-differentiation of neoplastic cells without cell death. NIH Grant Number P20 RR-16460 from the IDeA Networks of Biomedical Research Excellence (INBRE) Program of the National Center for Research Resources

IDeA Implementation and Outcomes

IDeA-O1. Leveraging INBRE funds: RISE-UP (Research Internships in Science of the Environment - University Program) an Undergraduate Research Mentoring Program at Arkansas State University

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Taylor Ingle, Arkansas State University

INBRE funds have been used to support a number of undergraduate researchers at Arkansas State University. Undergraduate researchers were important members of the research teams in several of the projects described in posters at this meeting and in other INBRE-funded projects at ASU. In the past 5 years over 50 undergraduate and student researchers have been involved in these and other INBRE-funded projects at ASU. To date, 18 of these students have graduated and are pursuing careers or training in SMET fields and 15 are members of underrepresented minorities. This participation provided the basis for a proposal to the URM program at the NSF to support additional minority undergraduate and high school student researchers. This project was awarded \$800,000 to support a 5 year program that began in Sept 2008. Now in its 2nd year, this program has supported a total of 17 undergraduate students and 6 high school summer research interns. All of these students work with a faculty research mentor and are members of underrepresented minorities. Student participants work with mentors from a wide variety of disciplines include microbiology, biochemistry, molecular biology, neurophysiology, synthetic organic chemistry, mathematical modeling, plant genetics. These students are working on a wide variety of projects ranging from neuroscience to synthetic chemistry to plant genomics. Examples of some of the projects include development of novel chemical treatments for glaucoma, genetic strategies to increase cellulase production in maize, accumulation and distribution of inhaled nanoparticles, development of novel fluorophores and ecotoxicology of nanomaterials. Student participants are involved in all aspects of the research, from experimental design and research planning to preparing and presenting results of their research at regional and international meetings and as co-authors of manuscripts. Student researchers were initially supported by: NIH NCRR grant P20 INBRE RR-16460. Participants are now supported by NSF DBI 731603.

IDeA-02. Faculty Development Core to Support Mentoring of Foreign Born Promising Junior Investigators

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Based on needs assessment conducted among junior faculty and institutional priorities, the leadership of our COBRE found the need for a distinctive faculty development core. This core has been operating for the last four years and has been instrumental to our recovery following Hurricane Katrina. The core supports mentoring and career development; it also explores novel concepts, approaches and methods and bring objectivity, planning, accountability, and scholarship to faculty development. Activities have been designed to meet the specific needs of our promising junior investigators (PJIs) who are predominantly foreign born graduates. Components of the core include an ongoing faculty development curriculum with a core set of competencies in the areas of research, administration, leadership, communication, and cultural competency; a structured pathway to assist mentors and mentees with designing, monitoring, and evaluating mentoring contracts, and provides Research Seminars, Invited Speaker Series, Work in Progress, Journal Clubs and workshops on grantsmanship, communications, lab management and the ethical conduct of research. In addition, the core designs and evaluates mentoring activities at different levels: peer review, scientific, career mentoring and external mentoring. A career development plan is designed for PJIs that includes specific targets, measurable outcomes and periodic evaluation. The faculty development program has become a model at LSU Health Sciences Center (LSUHSC) and a model for other COBRE programs in IDeA states. Evaluation of the core activities reveal that foreign born PJIs need mentoring and training tailored specifically to their needs. These needs include: acculturation, written and oral communications, ethical conduct of research, grantsmanship, and support of the mentor-mentee relationship. Eight of nine PJIs who entered the program are international science graduates. Eight out of nine remain on the faculty at LSUHSC and seven have become independently funded. Since July 1, 2006, 22 grants (federal, state, and private) have been awarded to COBRE participants, who have authored 152 peer reviewed publications of which 25% were co-authored by PJIs and their mentors. The presentation at the regional meeting will discuss our findings in detail and how to replicate our methodology elsewhere.

IDeA-03. Community Based Research and Education (COBRE) Core Facility

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J. Hall-Barrow, University of Arkansas for Medical Sciences
Edgar Garcia-Rill, University of Arkansas for Medical Sciences

Background and Objective: We established a network of 15 sites, with 10 more to be added within the year, using T1 lines to link telemedicine units with real-time teleconferencing and diagnostic quality imaging. Fifteen units were placed in neonatal Intensive Care Units and 10 more in other delivery sites. We carried out weekly combined obstetric and neonatal educational conferences to establish guidelines for the care of premature babies and other common pediatric illnesses with outlying clinicians caring for mothers and their newborns. Initial studies evaluated the impact of telemedicine on regionalization of newborn care, and physician and other caregiver satisfaction with the educational part of the program.

Methods: Patterns of delivery were assessed through a linked Medicaid database before and after the telemedicine initiative to determine if the most at risk neonates were transferred to the perinatal center for delivery. Additionally, clinician satisfaction with the educational conference, combined with translational educational sessions, broadcast through telemedicine to practicing clinicians was assessed. Results Survey results from practicing clinicians revealed that they would change their practice to conform to the educational guidelines established in the educational conferences. Medicaid deliveries at the perinatal center before and after the telemedicine initiative in 2003 are shown in the Table. Conclusions: Telemedicine is an effective way to translate evidence based medicine into clinical care when combined with a general educational conference. Patterns of deliveries appear to be changing so that those newborns at highest risk are being referred to the perinatal center. A telemedicine initiative dedicated to Emergency Depts. in the State will begin this year. Supported by NCRR award P20 RR20146.

Birthweight	2001 (%)	2002	2003	2004
500-1000 grams	27.6%	19.9%	31.4%	34.5%*
1001-1500 grams	32.7%	24.2%	29.7%	30.6%
1501-2000 grams	20.3%	14.8%	24.1%	20.0%
2001-2500 grams	8.0%	7.9%	8.2%	7.5%

Table. *p<0.05 after 2002

IDEA-O4. The Center for Translational Neuroscience, 5 years of progress

Edgar Garcia-Rill, University of Arkansas for Medical Sciences

Objective: Our aims were to establish 1) a Career Development Program with mentoring/funding for 5 projects and 6 promising investigators without a history of support, 2) a multi-disciplinary Center, a Pilot Study Program, and recruitment of two senior and three junior investigators to build the critical mass of researchers, and 3) two Core Facilities, Administrative and Experimental, to support research. All aims were met and exceeded. Methods: We developed a Career Development Program and Center so that promising clinician and basic scientists could be mentored to secure funding. Results: We have mentored and supported 10 promising investigators without a history of support. In addition to funding 5 projects and 6 investigators with designated Mentors, we provided access to Core Facilities to 4 other investigators, and assigned them Mentors to help guide their efforts. We awarded 5 instead of 4 pilot study grants and assigned Mentors to each recipient to improve output. We recruited 3 senior and 2 junior faculty and established 6 (instead of 2) new Core Facilities. We discovered two new cures, two novel treatments and one new mechanism, all with clinical implications. We generated >\$13 million in new awards, published >130 articles and >150 abstracts. In addition, the projects supported during the last five years produced a novel treatment for tinnitus (ringing in the ears) that relieves the symptoms in a majority of patients, a new use for a drug (already used for another disorder) that relieves the excessive reflexes induced by spinal cord injury, an effective new treatment for spatial neglect due to stroke, and a new mechanism for sleep-wake control that promises to lead to the development of a new class of anesthetics and stimulants. Discussion and Conclusions: The oversight of a dedicated external advisory committee and considerable institutional support were critical to our success, along with selfless

mentoring by dedicated senior scientists. Supported by NCRR award P20 RR20146.

IDeA-P1. A Nutrition and Cancer Center at Marshall University

W. Elaine Hardman, Marshall University
Piyali Dasgupta, Marshall University
Philippe Georgel, Marshall University
Donald Primerano, Marshall University
Vincent Sollars, Marshall University
Richard M. Niles, Marshall University

Epidemiology studies indicate that diet and lifestyle choices contribute to the development of cancer. The COBRE renewal from Marshall University, PI Richard Niles, will support the development of a Center focusing on study of the effects of diet and dietary components on the prevention, development and treatment of cancer. The four proposed projects range from basic science to clinical trials. For the most basic science project, Dr. Philippe Georgel will investigate the effects of sulforaphane (found in cruciferous vegetables) on epigenetic regulation in prostate cancer cells. Dr. Piyali Dasgupta will define the mechanism for suppression of lung cancer growth by capsaicin, the heat in peppers. Dr. Vincent Sollars will use a transgenic mouse model for myelogenous leukemia to assess the ability of omega 3 fatty acids to enhance differentiation in myeloid progenitor cells. Finally, a pilot clinical trial, lead by Dr. Elaine Hardman, will determine whether dietary supplementation with omega 3 fatty acids can be used as a strategy to slow the progression of indolent B-cell malignancies. An expanded Genomics and Bioinformatics Core, under the direction of Dr. Don Primerano, will provide state-of-the-art services not only for Nutrition and Cancer Center researchers but for other researchers in the region. Next generation sequencing equipment will soon be acquired for deep sequencing to detect epigenetic modifications and novel polymorphisms that confer cancer susceptibility. In addition, automated DNA sequencing, expression-based gene profiling, real time PCR instrumentation, bioinformatics and biostatistical support will be available. The studies proposed by these projects will enhance our knowledge base of diet effects on cancer formation, progression and treatment. Formation of a Nutrition and Cancer Center at Marshall University will gather a critical mass of scientists interested in the common theme of nutrition and cancer and provide mutual support and collaboration opportunities to these scientists. The multiple projects expected to be spawned by this center will provide high quality training opportunities for undergraduate, graduate and post-doc students and will increase the base for high tech jobs in West Virginia.

IDEa-P2. BioMolecular Sciences Network: An integrated research effort at the University of Puerto Rico

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The BioMolecular Sciences Network (BMSN) embodies the integration of the BioMolecular Sciences Resources (BMSRs) in Puerto Rico as part of the research and career development objectives of the Puerto Rico Alliance for the Advancement of Biomedical Research Excellence (PRAABRE). Our overall goal is to provide mentorship and scientific support to empower PRAABRE-supported investigators and students from primarily undergraduate institutions (PUIs) in the Island. We envision that fostering a concerted, integrated, and collaborative environment in Puerto Rico, catalyzed by the BMSN, will i) contribute to the expansion of scientific knowledge among researchers and students at PUIs and ii) create productive partnerships between these investigators (including their mentors and collaborators) and those directing active research endeavors and instrumentation facilities (the Core Facility Coordinators) within the two main PRAABRE mentoring institutions in the Island: the Río Piedras and Medical Sciences Campuses of the University of Puerto Rico (UPR-RP and UPR-MS), respectively. These goals will be achieved by the integration of researchers in the two major areas of the BMSN: genomics and proteomics. The integration of resources and scientific expertise available at the BMSN will empower researchers and augment their scientific capabilities, resulting in a better use of BMSRs and enhanced competitiveness. In addition, the foster interaction will result in a more active networking among facilities originally supported by NCCR and their members, which will impact the collaborative, consulting, training, and service capabilities for all members of the PRAABRE program and researchers in Puerto Rico.

Microbiology/Immunology/Inflammation

MI-O1. OspC is a dissemination-facilitating factor of *Borrelia burgdorferi*

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Borrelia burgdorferi, the spirochetal agent of Lyme disease, establishes a localized infection at the site of tick bite, and then disseminates to distal tissues of a mammalian host, eventually causing a persistent infection, if left untreated. The lipoprotein OspC is a critical virulence factor of *B. burgdorferi* required for the initial stages of mammalian infection. By creating OspC-deficient bacteria that were modified to increase the expression of heterologous lipoproteins, previous studies have shown that OspC has dual early protective and dissemination-promoting functions. To further dissect the role of OspC during the course of mammalian infection, a borrelial mutant carrying an OspC antigen

with an N-terminal 5-amino-acid sequence deletion was generated. The mutation did not increase the 50% infectious dose or reduce the tissue bacterial burden significantly in the murine host. However, the deletion greatly impaired the ability of *B. burgdorferi* to disseminate to remote tissues after subcutaneous/intradermal inoculation into mice. Taken together, the study indicates that OspC plays a critical role in dissemination of *B. burgdorferi* during mammalian infection.

MI-O2. Molecular analysis of *Trypanosoma cruzi* isolates obtained from raccoons in South Central Kentucky

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Trypanosoma cruzi is the protozoan parasite responsible for Chagas' disease, the leading cause of heart disease in Central and South America. Although *T. cruzi* has been isolated from a variety of wild mammals in the southern and southwestern United States, it has only recently been identified in raccoons and opossums from the state of Kentucky. Eighteen isolates of *T. cruzi* were successfully obtained from raccoon blood samples by hemoculture in liver infusion tryptose medium supplemented with 10% newborn calf serum and penicillin/streptomycin. The purpose of the present study was to use a molecular typing approach to determine the genotypes (Type I, or Types IIa-IIe) of 13 of the 18 isolates. DNA samples were prepared from each isolate using a Qiagen mini kit, and PCR amplification was performed using published primers for the 24S rRNA sequence (D71 and D72), the non-transcribed spacer of the mini-exon genes (TC, TC1, and TC2), the 18S rRNA sequence (V1 and V2), and the TCZ1 and TCZ2 primers that amplify a 188-base pair segment of the repetitive 195-bp nuclear DNA sequence of *T. cruzi*. Based upon the results of this analysis, all 13 isolates appear to be Type II, the genotype of *T. cruzi* that has been most commonly reported from raccoons in the southeastern part of the United States. The support of NIH Grant Number 2 P20 RR-16481 from the National Center for Research Resources is gratefully acknowledged.

MI-O3. Aldose Reductase Catalyzes Reduction of Lipid Oxidation Products and AGE Precursors in Cardiovascular Tissues and Protects Against Early Atherosclerotic Lesion Formation

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Atherosclerotic lesion formation and diabetes is associated with the accumulation of oxidized lipids and carbohydrates. Products of lipid oxidation, particularly aldehydes, and AGE accumulation stimulate cytokine production and enhance monocyte adhesion; however, their contribution to atherosclerotic lesion formation remains unclear. To test the hypothesis that inhibition of aldehyde removal by aldose reductase (AR), which metabolizes both free and phospholipid aldehydes, would exacerbate atherosclerotic lesion formation we examined atherosclerotic lesions, metabolism of HNE and methylglyoxal and AGE accumulation in *Akr1b3* (AR)(-/-)/*apoE*(-/-) and cardiospecific *Akr1b4* (rat AR) and *Akr1b8*-(FR-1) transgenic mice. In atherosclerotic lesions of apolipoprotein

(apo)E-null mice, AR protein was localized with macrophage-rich regions and its abundance increased with lesion progression. Treatment of apoE-null mice with AR inhibitors sorbinil or tolrestat increased early lesion formation but did not affect the formation of advanced lesions. Early lesions of AR(-/-)/apoE(-/-) mice maintained on high-fat diet were significantly larger when compared with age-matched AR(+/+)/apoE(-/-) mice. Pharmacological inhibition or genetic ablation of AR also increased the lesion formation in male mice made diabetic by streptozotocin treatment. Lesions in AR(-/-)/apoE(-/-) mice exhibited increased collagen and macrophage content and a decrease in smooth muscle cells. AR(-/-)/apoE(-/-) mice displayed a greater accumulation of the AR substrate 4-hydroxy trans-2-nonenal (HNE) in the plasma and protein-HNE adducts in arterial lesions than AR(+/+)/apoE(-/-) mice. Acetol was generated in hearts perfused with methylglyoxal and its formation was increased in Akr1b4- or Akr1b8-transgenic mice. Reduction of AGE precursors was diminished in hearts from AR-null mice. Diabetic AR-null mice accumulated more AGEs in the plasma and the heart than WT mice and deletion of AR increased AGE accumulation and atherosclerotic lesion formation in apoE-null mice. These observations indicate that AR-catalyzed reduction is an important pathway in the endothelial and cardiac metabolism of AGE precursors. AR is upregulated in atherosclerotic lesions and it protects against early stages of atherogenesis by removing toxic aldehydes generated in oxidized lipids and it prevents AGE accumulation and atherosclerotic lesion formation.

MI-O4. Macrophage microRNA-155 and atherosclerosis

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Atherogenesis is a chronic inflammation in which cholesterol-loaded macrophage foam cell formation is the obligatory step. The content and behavior of the macrophage foam cells in atherosclerotic plaque determine ultimate fate of a plaque, either remaining clinically silent or causing heart attack and stroke. Oxidized low-density lipoproteins (oxLDLs) interact with toll like receptor 4 (TLR4) on the surface of the macrophages and trigger an inflammatory cascade that leads to the release of chemokines and cytokines. This release results in the growth in size and decrease in stability of the atherosclerotic plaque. However, the signaling network initiated by the oxLDL-TLR4 interaction is not well depicted. MicroRNAs are short, non-coding RNAs that regulate gene expression post-transcriptionally. One microRNA, microRNA-155 (miR155), plays an important role in the macrophage inflammatory response to lipopolysaccharide (LPS), a potent gram-negative bacteria-derived TLR4 ligand. The purpose of this study is to investigate whether miR155 plays a role in the macrophage inflammatory response to oxLDL, the primary endogenous TLR4 ligand, and therefore possibly has an impact on atherogenesis. Our data show that: 1. oxLDL stimulates macrophage miR155 expression in a TLR4-dependent manner. Macrophage miR155 levels positively correlate with pro-inflammatory cytokine expression; 2. Lentiviral vector-mediated overexpression of miR155 in macrophages enhances their inflammatory response to oxLDL and LPS, and impairs cholesterol efflux (a critical process to prevent macrophage cholesterol accumulation); 3. MiR155 expression levels are increased in both mouse and human atherosclerotic lesions. The increased miR155 is mainly derived from the lesional macrophage foam cells. Collectively, these data suggest that macrophage miR155 is part of the positive feedback loop of oxLDL-TLR4 inflammatory pathway, leading us to expect that macrophage miR155 is atherogenic. Our future studies will further investigate the

atherogenic role of macrophage miR155 in vivo using mouse models and define the underlying molecular mechanism.

MI-O5. Periostin modulates fMLP-directed Neutrophil Migration in Intestinal Inflammation

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Inflammatory bowel disease (IBD), primarily includes Ulcerative Colitis (UC) and Crohn's Disease (CD), is collectively characterized by excessive neutrophil (PMN) migration, a damaged intestinal epithelium, aberrant cytokine production and over-activation of intestinal fibroblasts. Periostin is a matri-cellular protein that is produced by fibroblasts, up regulated during acute inflammation, and participates in fibrotic reactions. A recent study revealed that in the intestine, periostin surrounds the basal pole of the crypt epithelium similarly to the location of the intestinal fibroblasts. However, the role of periostin in intestinal inflammation is has not been defined. To better understand the interactions between PMN, periostin and epithelial cells, PMN adhesion, PMN migration bioassays, Flow Cytometry, and immunofluorescence were utilized. Our results show that periostin is expressed on the surface of PMN and antibodies to periostin can inhibit PMN adhesion to a variety of extracellular matrix proteins. Periostin is also expressed by T84 intestinal epithelial cells and substantially up regulated when such T84 cells are exposed to the potent inflammatory cytokine interleukin-6 (IL-6), which is heavily expressed in IBD. For migration experiments, T84 intestinal epithelial cells were cultured on 5.m monolayers on the under surface and intestinal fibroblasts cultured on the upper surface, displaying an in vivo like phenotype. Following co-exposure (T84 cells and intestinal fibroblasts) to IL-6, there was an inhibition of fMLP-directed PMN migration across both cells. However, when such cells were not exposed to IL-6, no inhibition of migration was observed. Treatment of freshly isolated PMN with purified periostin rescues the inhibition of migration observed following of IL-6 treatment. In human non-inflamed intestine, immunofluorescence experiments confirmed that periostin is lightly expressed at the basal pole, but also lightly expressed at apical pole of the crypt epithelium. In both CD and UC intestines, periostin is markedly increased in the aforementioned intestinal regions and also prominently present in the lamina propria areas. These data strongly implicate periostin as modulator of the interactions between PMN and epithelial cells and as essential component in the inflammatory pathways in the intestine.

MI-O6. Effects of Dietary Fish Oil as an Adjunct to Oral Hygiene for Periodontitis

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Background: Dietary n-3 polyunsaturated fatty acids (n-3 PUFA; fish oils) have shown immune modulating effects on host responses in chronic inflammatory diseases. These affects encompass alterations in the production of various inflammatory lipid media-

tors, eg. prostaglandins, as well as associated cytokine and chemokine production by various host cells when stimulated. Periodontitis is a chronic oral infection with a broad array of bacterial genera and species that exist in biofilms juxtaposed to host tissues in the oral cavity. The qualitative and quantitative distribution of these oral bacteria trigger a chronic inflammatory response in gingival tissues and leads to loss of function, that is undermining the epithelial barrier, destroying underlying connective tissue, and eventually alveolar bone resorption necessary for maintenance of the supporting structures for the teeth. While periodontitis has various biologic processes that underpin the tissue destruction, generally treatment for the disease has focused on a mechanical intervention to physically decrease the microbial burden that triggers the disease. Thus, there is limited information, on clinical changes using biologic modifiers of host responses, including n-3 PUFA as an adjunctive treatment of periodontal disease. Objective: This study evaluated changes in clinical measures and local/systemic markers of periodontal disease in an ongoing, placebo-controlled, clinical study of 78 subjects randomized to either oral hygiene instruction (OHI) +Placebo or OHI + n-3 PUFA (1g/tid p.o.). Clinical measures, serum, and GCF samples were collected at baseline, 8, 16 and 28 weeks. The fluids were evaluated for a range of inflammatory mediators and serum IgG antibody to a battery of oral pathogens. Results: n-3 PUFA showed a statistically significant adjunctive improvement over OHI alone for full mouth mean attachment loss (CAL), but not for probing depth (PD), or bleeding on probing (BOP). Periodontitis sites (=5mm PD, =3mm CAL, =2 BOP) showed significantly lower GCF levels of IL-8, IL-6, and PGE2 in the OHI+PUFA group versus OHI alone. Conclusions: Initial outcomes suggest dietary n-3 PUFA may provide adjunctive clinical benefit to OHI as measured by clinical periodontal measures and host response parameters in periodontitis. Supported by NCRR grant P2ORR020145.

MI-P1. Dengue virus transmission dynamics are characterized by competitive interactions between naturally coincident serotypes

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This work delves into the important parameters of the vectorial capacity (VC) equation in an effort to better model the transmission of dengue in the context of co-circulation of multiple serotypes. Dengue virus is one of the most important arboviruses in the world; and it is the leading cause of hospitalization of children in Southeast Asia. Four antigenically distinct serotypes (1-4) are known to co-circulate in Southeast Asia. The virus is transmitted by the mosquito vector *Aedes aegypti* which becomes infected after taking a blood meal from an infected human. After infection, the virus must escape the midgut and disseminate to the salivary glands in order to be transmitted successfully by the mosquito. Dissemination into the legs (and thereby the salivary glands) is used to measure vector competence (b) of the mosquito; that is, the ability of the mosquito to acquire and transmit the virus. The time it takes, in days, for the virus to disseminate is the extrinsic incubation period of the virus (N). These two parameters, b and N , are important in the calculation of VC, a measure of transmission cycle efficiency. While much work has been done on dengue type 2 viruses in comparing the EIP and vector competence of strains, little has been done to characterize the differences between dengue serotypes. In light of the fact that dengue is found in complex urban ecologies involving up to four co-transmitting serotypes, it is an over-

simplification to estimate transmission without accounting for efficiency differences between serotypes.

MI-P2. Molecular mechanisms of *Moraxella catarrhalis* resistance to antimicrobial peptides

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Moraxella catarrhalis is the third leading cause of otitis media accounting for 15-20% of the cases in the United States. A common feature of otitis media is the rapid neutrophil influx early during infection. These phagocytic cells are key components of the innate immune system by destroying microbes in part through the production of cationic antimicrobial peptides (CAMPs). One of our aims is to identify and characterize *M. catarrhalis* genes involved in resistance to CAMPs. To this end, we generated a transposon mutant library and screened it for sensitivity against the CAMPs polymyxin B and neutrophil derived LL-37. Sequences from the cloned DNAs bordering the transposons were searched against the GenBank database. Several genes were identified that may play a role in *M. catarrhalis* defense against polymyxin B and LL-37. We are currently investigating the global transcriptional response of *M. catarrhalis* to these peptides.

MI-P3. *P. gingivalis* and *F. nucleatum* induce HIV-1 reactivation in monocytes/macrophages through TLR2 and TLR9 activation

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Translocation of bacteria or their products (e.g. LPS and DNA) from mucosal surfaces to systemic circulation appears to be associated with increased HIV-1 replication from latently infected cells, antiretroviral therapy failure and AIDS progression in HIV-1+ patients. Similar to the gut, the oral cavity is colonized by a diverse number of commensals and opportunistic pathogens, which overgrow and cause disease upon immunosuppression early after HIV-1 infection. Although oral co-infections (e.g. periodontal disease) are highly prevalent in HIV-1+ patients and appear to positively correlate with viral load levels, the potential for oral bacteria to induce HIV-1 reactivation in latently infected cells has received little attention. Using BF24 monocytes/macrophages stably transfected with the HIV-1LTR promoter driving CAT expression, and THP89GFP cells, a model of HIV-1 latency, we sought to determine the ability of oral bacteria, including Gram-negative *P. gingivalis* and *F. nucleatum*, and Gram-positive *S. gordonii* and *S. sanguinis* to induce HIV-1 reactivation by CAT-ELISA, fluorescence microscopy, flow cytometry and fluorometry. Levels of p24 and pro-inflammatory cytokines in supernatants were determined by ELISA. The oral Gram-negative but not Gram-positive bacteria enhanced HIV-1LTR activation in BF24 cells. Using purified TLR2, TLR4 and TLR9 agonists, as well as neutralizing antibodies and antagonists, we demonstrated that TLR9 engagement by *F. nucleatum* and TLR2 binding by both Gram-negative bacteria are involved in HIV-1LTR activation; however, TLR4 activation had no effect. Use of NF.Β or Sp1 specific

chemical inhibitors suggested that these transcription factors are positive and negative regulators of bacterial-induced HIV-1LTR activation, respectively. HIV-1LTR activation and viral replication were similarly induced by oral bacteria in THP89GFP cells. Detectable levels of TNF α , but no IL-6, were induced by Gram-negative bacteria and neutralization of TNF α reduced HIV-1 reactivation. These results suggested that TLR2 and TLR9 activation by oral Gram-negative bacteria, *F. nucleatum* and *P. gingivalis*, as well as TNF α produced in response to these stimuli enhance HIV-1 reactivation in monocytes/macrophages. Increased bacterial growth and emergence of periodontal pathogens or their products accompanying chronic oral inflammatory diseases could be risk modifiers for viral replication and transmission, systemic immune activation and AIDS progression in HIV-1+ patients. Supported by NCRR grant P20RR020145.

MI-P4. Virion Particles of Influenza and Respiratory Syncytial Virus Directly Modulate Human Natural Killer Cell Activity

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Natural killer (NK) cells are one of the first immune cells to reach the site of viral infection, and are capable of exerting potent anti-viral effects. Pathogenic viruses, in turn, have evolved mechanisms for escaping or suppressing the NK cell response. This work focuses on the interaction between human NK cells and virion particles of human respiratory syncytial virus (HRSV) and influenza A virus, with the goal of distinguishing between protective and pathological responses triggered by virion proteins. Expression of immunologically relevant inflammatory mediators, cytokines, and cell surface molecules by the human NK-92 cell line was characterized by real-time polymerase chain reaction (RT-PCR) following two hour exposure to UV-inactivated virus. Virion exposure modulated the expression of several immunologically relevant genes in NK cells. HRSV uniquely promoted an allergic-type inflammatory response by boosting interleukin (IL)-5 and IL-13 expression. Exposure to influenza A virus enhanced IL-17A expression. High expression levels of this cytokine are associated with pathological inflammation. Both HRSV and influenza A virus altered the expression of other immunologically relevant genes such as colony stimulating factor I and OX40 ligand. Altered expression of these immune response mediators in vivo would impact the course of infection and the effectiveness of innate and adaptive immune responses. Future work will more specifically investigate the cause of the immunomodulation, which will include virion fractionation and testing of isolated viral glycoproteins. Signaling molecules involved in generating the NK cell response will also be investigated. Understanding the immunomodulatory potential of virion components will yield insights into viral pathogenesis and allow the design of more effective subunit vaccines.

MI-P5. Expression of Cytokines and Chemokines in the Genital Tract of Stressed Chlamydia Infected Mouse Model

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Genital infection by *Chlamydia trachomatis* (CT) is the most common bacterial sexually

transmitted disease (STD) worldwide, with an estimated 4-5 million Americans infected yearly. This disease causes serious reproductive health complications in women, including pelvic inflammatory disease (PID), ectopic pregnancy and infertility. There is growing evidence that stress is implicated as a risk factor for various infections, but its effect on chlamydia genital infection and complications has not been well defined in animal models. Recent studies in our laboratory demonstrated that physical or psychological stress application to mice resulted in increased susceptibility to genital *Chlamydia trachomatis* infection. The cytokine and/or chemokine responses in this animal model remain to be illustrated. The objectives of this study were to determine if cold water-induced stress can result in alterations the expression of cytokines and/or chemokines in the genital tract of *Chlamydia* infected mouse model. Mice were stressed by immersing in cold water for 5 minutes for 8 days. Mice were infected intravaginally with approximately a million IFU per mouse. Genital tracts were harvested and using RT-PCR the messenger expression of selected proinflammatory and chemokines was quantified at 48 hour after infection. Gel image analysis showed stress markedly decreased expression of interleukin (IL-1..) L-6 and TNF-.alpha, RANTES and toll-like receptor (TLR)-2. In contrast, the mRNA levels of IP-10 and TLR-4, were elevated in the genital tract of stressed mice compared to the control. Our data suggest that stress may lead to differential production of proinflammatory cytokines, chemokines and toll-like receptors that may have key roles in coordinating host's responses to inflammation and chlamydia genital infection.

MI-P6. Rnd3 promotes barrier recovery by inhibiting Rho

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Rnd3, recently implicated as an endogenous inhibitor of Rho signaling, is present in cultured endothelial cells but its functional significance is unknown. We tested the hypothesis that Rnd3 inhibits Rho signaling in endothelial cells during an inflammatory challenge. We assessed in vivo expression of Rnd3 in rat mesenteric microvessels by immunofluorescence confocal microscopy. RhoA activation was assessed in human umbilical vein endothelial cells (HUVEC) treated with thrombin (1U/ml) for 5, 30, or 60 min. using a commercially available G-LISA kit. HUVEC were mock-transfected or transfected with pMAT-FLAG-Rnd3, Rnd3 siRNA, or siCONTROL RNA. The results show that Rnd3 is present in vivo within endothelial cells. Thrombin significantly increased Rho activity in all groups treated, however the time course varied, particularly at 60 min. Rho-GTP levels were significantly lower in pMAT-FLAG-Rnd3 cells compared to mock at 60 min. In contrast, in Rnd3 siRNA cells, Rho-GTP levels at 60 min. were significantly higher than siCONTROL cells. The data indicate that Rnd3 accelerates termination of thrombin-induced Rho activation, which may serve as an important negative feedback signal for concluding inflammatory signaling in endothelial cells. Supported by NIH RR018766 and a grant from the American Heart Association.

MI-P7. Differences in Electrostatic Properties at Antibody-Antigen Binding Sites - Implications for Specificity and Cross-Reactivity

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The neolactoseries, embryonic Lewis Y (LeY) antigen is a tumor associated carbohydrate determinant that plays an important role in tumor growth, progression, and metastasis. Anti-LeY monoclonal antibodies are proposed as immunotherapeutics and are in the clinic. Structural studies of monoclonal antibodies targeting the Lewis Y antigen therefore are important to provide a clearer understanding of structure/function relationships relevant to their clinical use. Several anti-LeY antibodies have been described in the literature for clinical application. Comparing the BR55, hu3S193, BR96 and B3 antibodies bound to LeY, we observe that while these antibody-carbohydrate complexes employ similar amino acid residues to participate in intermolecular hydrogen bonding with the LeY determinant at the binding site, the ensemble of epitopes on the LeY determinant that the antibodies recognize can be different. This emphasizes the finer specificity of these antibodies despite recognizing the same tumor associated antigen. We have also examined the conformational differences between the free and bound anti-LeY antibodies. Comparative molecular dynamics (MD) simulation was performed with and without the LeY antigen, starting from the initial crystal structure or in some cases the modeled structure of the antibodies. The potential energies from the production dynamics clearly showed marked difference in the bound antibody from its unbound form indicating that the bound form restricts the conformational mobility of the respective antibodies. This disparity suggests a conformational readjustment process linked to antigen binding in altering the fine specificity of these antibodies. We have also shown that these antibodies have a common germline after which they have acquired different somatic mutations in their hypervariable regions that resulted in distinctly different affinity matured antibodies. While the antibodies converge on recognizing the same determinant, they do so in different ways. Our work demonstrates that higher electrostatics, both as a number of short-range electrostatic interactions and their contributions leads to higher binding specificity in the matured antibodies.

Neuroscience

NS-O1. Neuroprotectin D1 (NPD1) as a Sentinel for Neurodegenerations

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The significance of the selective enrichment and avid retention of omega-3 essential fatty acid (docosahexaenoyl –DHA – of phospholipids) in the CNS has remained, until recently, incompletely understood. We and our colleagues discovered neuroprotectin D1 (NPD1, 10R, 17S-dihydroxy-docosa-4Z, 7Z, 11E, 13E, 15E, 19Z hexaenoic acid), a docosanoid derived from a 22C precursor (DHA) (unlike eicosanoids from the 20 C arachidonic acid). NPD1 is made in response to oxidative stress and brain ischemia-reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in brain damage, oxidative-

stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid- β peptide. Thus, we envision NPD1 as a protective sentinel, one of the very first defenses activated to sustain cell homeostasis during initiation and early progression of neurodegenerations: 1) Photoreceptors renew membranes and DHA via shedding of their tips and phagocytosis by retinal pigment epithelial (RPE) cells. Photoreceptors are in an oxidative stress-prone environment. We show that phagocytosis of photoreceptors promotes via NPD1 synthesis specific refractoriness to oxidative stress-induced apoptosis, which in turn fosters homeostatic photoreceptor cell integrity. Disruptions of the sentinel role of NPD1 in photoreceptor renewal may participate in macular degeneration leading to blindness. 2) NPD1 is reduced in CA1 areas from Alzheimer's patients. Thus, we have explored NPD1 in cellular models that recapitulate Alzheimer's pathology. Human neurons and astrocytes challenged by amyloid- β or by overexpressing APP^{Sw} (double Swedish mutation) show that NPD1 downregulates amyloidogenic processing of amyloid- β precursor protein, switches off pro-inflammatory gene expression (TNF- α , COX-2 and B-94-TNF- α inducible pro-inflammatory element), and promotes neural cell survival. The apoptotic cascade involves multiple checkpoints. NPD1 regulation targets upstream events of apoptosis as well as neuroinflammatory signaling, in turn promoting homeostatic regulation of cell integrity. Supported by NINDS R01 NS046741, NEI R01 EY005121 and NCRR P20 RR016816, and NCRR COBRE P20 RR016816 "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience.

NS-O2. Impairment of hippocampal oscillatory activity in early epileptogenesis

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Epileptogenesis is a dynamic process involving several mechanisms that lead to a permanent state of spontaneous brain hyperactivity. Precursors of lipid mediators, cleaved by specific phospholipases upon activation by neurotransmitters (such as glutamate) and other neuromodulators, play a crucial role in the genesis of seizures. Synaptic membranes are richly endowed with docosahexaenoic acid (DHA), an omega-3 essential fatty acid. DHA is the precursor of NPD1. Seizure or early cell injury induce the synthesis of NPD1 (10R, 17S-dihydroxy-docosa-4Z, 7Z, 11E, 13E, 15Z, 19Z-hexaenoic acid). The goal was to determine the critical oscillatory components that participate in the early stages of epileptogenesis and determine the role of NPD1 in the hippocampal oscillations. Limbic status epilepticus (SE) was induced by kainic acid or pilocarpine in C57/BL6 adult mice. Silicon probes with 16 channels (100 μ m, spacing) were implanted in the dorsal hippocampus. The signal was amplified and digitized using multi-acquisition systems in order to obtain local field potentials (LFP) and spike units. Number of spike trains, frequency bands and oscillation patterns were analyzed. Racine's score and LFP were used to quantify seizure severity at different time points after SE. At the end of the experiments, brain samples were collected for morphology analysis. Spontaneous epileptiform, interictal-like spikes and high frequency events were observed within two weeks after SE. These events were associated with a progressive disruption of the physiological voltage-versus-depth profile of the hippocampal layers field activity. Also, preliminary data showed NPD1 modified frequency and duration of HFO events. These observations suggest that during the early stages of epileptogenesis physiological os-

cillatory patterns are disrupted before the onset of clinical seizures where HFOs work as a kindling-like process that promotes development of chronic limbic seizures. This disruption may continue to progress, impair inhibition and promotes seizure susceptibility in temporal lobe epilepsy. (NCRR, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"; PI: Nicolas Bazan).

NS-O3. Assessing the Role of Molecular Chaperons CCT/PhLP1 in Photoreceptor Neurons

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The eukaryotic cytosolic chaperonin-containing TCP-1 (CCT; also known as TRiC), and its cofactor phosducin-like protein 1 (PhLP1) have been proposed to play central role in folding of heterotrimeric G proteins. We confirmed expression of CCT and PhLP1 in the photoreceptors that produce large amount of heterotrimeric G protein, transducin, required for their function and viability, and explored physiological significance of these chaperones for visual function. The activity of CCT/PhLP1 was suppressed using transgenic expression of the N-terminally truncated dominant-negative mutant, PhLPs, in the photoreceptors. Retinal function and anatomy was monitored using electroretinography and light microscopy. Expression levels of protein were determined by Western blotting and quantitative RT PCR. We generated several transgenic mouse lines characterized by different expression levels of PhLPs. Each of these lines was found to have retinal degeneration. Mice expressing higher levels of PhLPs failed to fully develop photoreceptors and sustained massive photoreceptor loss by postnatal age 12 days (P12). At the same age, photoreceptors expressing less PhLPs appeared to develop normally, however their loss became apparent by age P25. The analysis of protein expression levels revealed that, prior to the onset of massive photoreceptor death, the level of the alpha and gamma subunits of transducin were profoundly reduced. Several other visual signaling and structural proteins, including rhodopsin, phosducin, and ROM1, were also down-regulated, in contrast to PDE6, and beta-tubulin, whose levels remained unchanged. Our studies provide new insights into the role of molecular chaperones in vertebrate photoreceptors and reveal a new type of retinal degeneration caused by their malfunction. We identified visual heterotrimeric G protein, transducin, as a major client of chaperonin CCT and putative culprits of photoreceptor degeneration.

NS-O4. Endoplasmic Reticulum Stress Precedes Onset of Photoreceptor Degeneration in the Ccl2^{-/-} / Cx3cr1^{-/-} Mouse Model of AMD

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Purpose: Vision loss by age-related macular degeneration (AMD) is the foremost cause of blindness in the US. The double knockout mouse (Ccl2^{-/-}/Cx3cr1^{-/-}) exhibits macrophage accumulation, drusen-like deposits, lipofuscin, Bruch's membrane thickening, RPE atrophy, subretinal capillary growth, and photoreceptor loss (Ambati et al., 2003), all

of which are characteristics of AMD. This study tests the hypothesis that photoreceptor apoptotic cell death in this AMD model may result as a consequence of excessive ER stress. Methods: The Ccl2^{-/-}/Cx3cr1^{-/-} mice and the C57Bl/6 control mice were maintained in our animal colony under normal conditions. Eyes from age-matched control and transgenic animals, 2- and 18-months-old, were prepared for cryo-sectioning and immunohistochemistry by standard methods, and the ER stress marker GRP78-BiP was immunolocalized in vertical retinal sections cut from the superior margin through the optic nerve to the inferior margin. Images of the immunolabeled sections were obtained by laser-scanning confocal microscopy. Results: These sections demonstrated increased expression of this ER stress marker in inferior retina (the area first to demonstrate fundus changes and loss of structure). Superior retina maintained a greater degree of structural integrity, but expressed higher levels of ER stress marker. Retinal sections of control C57Bl/6 mice displayed only low expression of this marker. In 2-month-old Ccl2/Cx3cr1-deficient mice, retinal damage has already begun in the inferior retina with some areas demonstrating loss of structural integrity. Even at 2 months, these mice demonstrate labeling with the ER stress marker. Conclusions: Regions of the retina that had not yet undergone degeneration exhibited the highest levels of the ER stress marker, while age-matched control retinas maintained only low levels of label. This demonstrates that ER stress occurs prior to photoreceptor loss, and, therefore, suggests that the unfolded protein response is triggered within the photoreceptors at an early time, leading to ER stress and photoreceptor apoptosis. Thus, early stress events leading to AMD may be possible targets for pharmacological intervention. Support: NCR, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"; PI: Nicolas G. Bazan.

NS-05. Carboxyl methylation of proteins is essential for survival and function of photoreceptor neurons

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Carboxyl methylation (CM) at the C-termini of proteins is a critical step in the post translational lipid modification referred to as prenylation. The importance of CM is underscored by embryonic lethality observed in mice defective in CM. While it is clear that the addition of a prenyl group is needed for association of proteins with membranes, the significance of the additional smaller methyl group is not obvious. It is thought that methylation, a reversible modification, may enhance the hydrophobicity of proteins modulating their association with membranes. Alternatively, methylation has been proposed to assist in assembly of multimeric signaling proteins. Several proteins in photoreceptor neurons, particularly in the outer segments that contain proteins involved in light signal transduction are methylated. These include heteromeric proteins such as transducin and phosphodiesterase or monomeric rhodopsin kinase and a group of small GTPases. To evaluate the role of this protein modification in the survival and function of the retinal neurons, we specifically disrupted carboxyl methylation in the developing retina at embryonic day 9. Surprisingly, retina lacking CM are able to develop normally up through postnatal day eight (P8) with no observed cell death. However at P10, when ciliated photoreceptor outer segment development begins, apoptosis is evident in the photoreceptor neuronal layer of the retina lacking CM. Even though photoreceptor nuclei can be observed in P14 sections, rod photoreceptor neurons do not function as measured by light evoked potentials recorded by electroretinogram (ERG).

Our studies conclusively demonstrate that methylation is critical for proper functioning and stability of photoreceptor neurons. Our preliminary results suggest a defect in morphogenesis of ciliated photoreceptor outer segments implicating the importance of methylation in functioning of small GTPases and the formation of cilia. Future studies will explore how CM affects the function, membrane association of small GTPases and role of CM in assembly of heteromeric signaling protein complexes.

NS-O6. A Novel Three-dimensional Imaging Method for Analyzing Retinal Degenerative Mechanisms of Choroidal Neovascularization

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PURPOSE: To remediate the deficiencies of the two most common CNV imaging methods, RPE-Choroid flatmounts and frozen sections, by developing a method of 3D imaging for the complete CNV lesion complex. **METHODS:** Laser CNV was induced in male C57Bl/6 mice (50 μ m dia, 100 msec, 150 mW) and eyes collected 14 days later. Eyes were either cryosectioned (20 μ m) and immunolabeled conventionally or prepared as eyecups, de-pigmented, immunolabeled, embedded in Spurr's resin and serial-sectioned at 100, 500, or 995nm thickness; some serial-section eyes were stained with toluidene blue. Eyes were immunolabeled for vascular endothelial cells (GS-IB4), microglia (CD11b), glutamine synthetase, and/or nuclei. Images were acquired on a confocal microscope and three-dimensionally reconstructed. **RESULTS:** Multiple fluorescent labels were detected as deep as 1mm in resin-embedded eyes imaged directly from the block. Signal detection through RPE was limited to the surface of the first melanin granule layer. Melanin granule de-pigmentation required bleaching, resulting in antigenicity loss. Fluorescently stained nuclei, short (405nm) and long (633nm) wavelength excitation, were detectable in semi-thin plastic sections as thin as 100nm with a signal equivalent to optical sectioning methods. Affine transformation alignment of 995nm thick toluidene stained serial-sections resulted in minimal mismatch, permitting 3D reconstruction. **CONCLUSION:** Three-dimensional reconstructions are commonly achieved using confocal microscopy but melanin-dense RPE in CNV lesions rapidly extinguishes signal detection thereby thwarting this approach. While not new, serial-section techniques are typically only used with bright-field or electron microscopy. These results demonstrate that fluorescent immunolabeling techniques are compatible with the plastic embedding and semi-thin sectioning used in serial-section technique. Further, we demonstrate that a high-fidelity 3D reconstruction of the entire CNV lesion complex, with retina-choroid integrity maintained and immunofluorescent labeling, is attainable using serial-section technique. This method will provide a new level of analysis, that can clarify the complex and controversial signaling/cellular events involved in CNV. **SUPPORT:** NCRN, COBRE P20 RR016816, Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience; PI: Nicolas Bazan.

NS-07. Cortical networks for sound recognition in the congenitally blind

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Introduction: How do the cortical systems for hearing perception compare between sighted and blind individuals? Using functional magnetic resonance imaging (fMRI), we previously reported that the bilateral posterior middle temporal gyri (pMTG) are activated when sighted participants hear and recognize every-day non-verbal action sounds (Lewis et al., (2004) *Cerebral Cortex* 14:1008). These cortical regions are situated midway between auditory and visual cortices, and are responsive to visual motion. Thus, the pMTG foci might have represented some form of “visual imagery” associated with the sounds. To test this possibility, we performed this same listening paradigm in blind participants, who have never had visual experience.

Methods: Using 3T fMRI, we imaged congenitally blind participants (n=10) and age-matched sighted participants (n=18) while they listened to 105 recognizable forward-played sounds (1.1-2.5sec), 105 unrecognizable backward-played versions of those sounds, and 140 silent events. Participants indicated if they could (1) recognize the sound, (2) were uncertain, or (3) could not recognize the sound. Using AFNI software, BOLD brain responses were analyzed based on each individual’s judgment responses. A t-test revealed group-average results.

Results: In both groups, successful recognition of forward-played action sounds, relative to hearing the corresponding unrecognized backward-played sounds, evoked activation along partially overlapping portions of the left and right pMTG ($p < 0.01$, uncorrected). Moreover, the congenitally blind group uniquely revealed substantial activation along portions of occipital cortex, located posterior to the pMTG bilaterally.

Conclusions: The pMTG activation foci for action sound recognition in the sighted may effectively be translated along the cortical mantle further posterior into occipital cortex in the blind, extending well into what would have been visual cortex. The moderate degree of overlap of bilateral pMTG regions between the sighted and blind groups suggested that visual input is not required for establishing the functional roles of these regions. Rather, the pMTG regions appear to function by generating abstracted or “supramodal” representations of object and/or sound-source action knowledge, independent of whether or not a person has the sense of vision (or hearing). Additionally, these higher-level representations of action knowledge may govern re-organizational mechanisms in cortex.

NS-08. Effects of maternal smoke exposure on nAChR subunit mRNA expression in brains of peripubertal mice

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Maternal smoking is associated with a wide variety of cognitive, physiological and behavioral deficits in resulting offspring. Some of these deficits are noticeable at birth, others are not detectable until puberty. While it is known that several neuroactive components of cigarette smoke cross the placenta, the mechanisms by which prenatal exposure to nicotine and the other constituents of cigarette smoke may produce these deficits are not well understood. Previous studies have shown that regional and temporal concentrations and turnover rates of monoamine neurotransmitters (serotonin and dopamine) are affected by maternal smoking. The experiments described herein establish the spatial and temporal gene expression patterns of the nicotine-sensitive acetylcholine receptor (nAChR) subunits in the developing brain of pups exposed to the constituents of cigarette smoke in utero. Quantitative realtime reverse transcription PCR (qRT-PCR) was used in establishing relative mRNA concentrations of selected nAChR subunits in several regions of the brain (hippocampus, hypothalamus, frontal cortex, and striatum) before, during and after puberty. The levels of mRNAs for $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ subunits in pups born to dams that were exposed to cigarette smoke during pregnancy (350 ml of smoke from 3R4F cigarettes, 3 times per day, 15 min per exposure from gestational day 4 through parturition) were compared to those measured in pups born to unexposed dams. mRNA levels were measured on PND 60. Total RNA extracted from brain tissue reverse-transcribed by oligo(dT) priming synthesized the cDNA template for realtime qPCR and was detected with iQ SYBR Green. Primers targeting the mouse nAChR subunits- $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ and gap junction protein, delta 2 (Gjd2) generated amplicons ranging from 69 (Gjd2) to 308 ($\alpha 3$) bp. nAChR subunit and connexin mRNA concentrations were normalized to several housekeeping genes including mouse β -actin, beta-2 microglobulin (B2m), pgk-1, glucuronidase, beta (Gusb), gap and 18s and analyzed using REST (Relative Expression Software Tool) software and a pair wise fixed reallocation randomization test. Preliminary results suggest no differences in hippocampal nAChR $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ subunit mRNAs between PND 35 pups born to exposed and unexposed dams. However, differences in $\alpha 5$ in the frontal cortex on PND 60 have been observed. Supported by: NIH NCRR grants P20 INBRE RR-16460 and the: Arkansas Biosciences Institute.

NS-O9. Gene Expression of Regulators of Serotonin in Dorsal Raphe Neurons of Subjects Diagnosed with Depression

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The relationship between serotonin (5HT) and major depressive disorder (MDD) is much studied but certain critical aspects are still ambiguous. Given the evidence that 5HT neurotransmission is reduced in depressed subjects, it is possible that one or more of the 5HT regulators may be altered in the dorsal raphe (DR) of depressed subjects. Candidates that regulate 5HT synthesis and neuronal activity of 5HT neurons include intrinsic regulators such as the rate-limiting enzyme tryptophan hydroxylase 2 (TPH2), 5HT autoreceptors, 5HT transporter and transcription factors, as well as, afferent regulators such as estrogen and BDNF. The present study was designed to quantify mRNA concentrations of above 5HT regulators in an isolated population of 5HT-containing DR neurons of MDD and gender-matched psychiatrically normal control subjects. We found that mRNA concentrations of the 5-HT1D receptor and the transcription factors, NUDR and REST were significantly increased in the female MDD subjects compared to female control subjects. No significant differences were found for these and any of the other transcripts in the male MDD subjects compared to male controls. This study reveals sex-specific alterations in gene expression of the presynaptic 5-HT1D autoreceptors and the 5HT-related transcription factors, NUDR and REST in DR neurons of women with MDD.

NS-O10. Reduced Level of Metabotropic Glutamate Receptor 5 (mGlu5R) Protein in the Prefrontal Cortex in Major Depressive Disorder

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Clinical, postmortem and preclinical research strongly implicates the dysregulation of glutamatergic neurotransmission in major depressive disorder (MDD). Our recent postmortem studies have demonstrated reduced expression of specific subunits of the ionotropic glutamate N-methyl-D-aspartate receptor (NMDAR) in the prefrontal cortex (PFC) in MDD. This coincides with clinical reports showing antidepressant properties of NMDAR antagonists in depressed individuals. Along these lines it has been observed that group I metabotropic glutamate receptors (mGluRs) mGlu1R and mGlu5R antagonists exhibit antidepressant-like activity in animal screening procedures. The mGluRs have not been previously examined in postmortem brain tissue from subjects diagnosed with MDD. Thus, the aim of this study was to examine protein and mRNA expression of mGlu1R and mGlu5R in the PFC from depressed subjects using Western

blot and quantitative real time RT-PCR methods. Prefrontal samples were obtained from 14 depressed and 14 psychiatrically healthy controls that were matched for age, gender and postmortem interval. The results revealed that there was reduced expression of the mGlu5R protein, with no changes in mRNA, in the PFC of depressed subjects relative to controls ($p=0.005$). In contrast, the level of mGlu1R protein was unchanged in the same depressed subjects. Since protein expression of mGluRs may be influenced by antidepressant exposure we have investigated the mGlu5R immunoreactivity in the PFC of male Rhesus monkeys treated with fluoxetine (dose escalated to 3mg/kg, p.o; $n=7$) or placebo ($n=6$) for 39 weeks. The expression of mGlu5R protein was unchanged in the PFC of monkeys treated with fluoxetine as compared to the placebo group. Our findings provide the first evidence that the protein level of mGlu5R is reduced in the PFC in MDD. This observation is in line with novel neuroimaging findings showing markedly reduced binding to mGlu5R in the PFC in living depressed patients. Based on our finding it could be cautiously inferred that the observed reductions in mGlu5R in depressed subjects are unlikely the effect of antidepressant exposure prior to death. Taken together, these studies further highlight the involvement of mGlu5Rs in the pathophysiology of MDD and demonstrate that mGlu5Rs are uniquely suited as targets for the discovery of novel antidepressant medications.

NS-O11. The ras homolog rhes affects regulation of adenylyl cyclase by dopamine receptors

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Rhes is an intermediate size GTP binding protein highly enriched in striatum. It has recently been shown to be critical to the region-specific loss of neurons in Huntington's Disease [Subramaniam et al., *Science* 324(2009) 1327-1330]. However, very little is known about its physiological functions. We have previously shown that Rhes is involved in regulating dopamine systems. For example, rhes mRNA and protein are decreased upon dopamine denervation [Harrison et al., *Brain Research* 1245(2008) 16-25]. Behaviorally, loss of Rhes in mice results in increased dopamine-mediated stereotypy but decreased dopamine D1 receptor-mediated grooming [Quintero et al., *NeuroReport* 19(2008) 1563-1566]. This finding led to the hypothesis that Rhes normally inhibits dopamine signaling through Gas/olf pathways (as in stereotypy), but promotes signaling through Gαq/11 pathways (as in grooming). In order to test this hypothesis, we have begun to investigate Rhes effects on dopamine signaling at the cellular level. Primary striatal cultures from Rhes wild type (WT) and knockout (KO) mice were used for measuring Rhes effects on cAMP accumulation as a test for activation of the adenylyl cyclase (AC) pathway. Cultures were prepared from E15-17 fetuses and used after 8-10 days in vitro. Both WT and KO cultures express D1 and D2 mRNA, as determined by RT-PCR. However, only WT cultures express rhes mRNA and protein, determined by RT-PCR and Western blotting, respectively. Treatment of cultures with the D1 agonists SKF81297 or SKF83822 (40 minutes) resulted in increased cAMP production, but this effect was enhanced in KO cultures compared with WT, suggesting that Rhes normally inhibits this response. However, inhibition of forskolin (25 μM)-stimulated cAMP by the D2 agonist quinpirole was compromised in KO versus WT cultures ($p<0.05$, $n = 3$). Pull-down assays with a His-tagged Rhes protein showed interaction of Rhes

with Gai and Gao, but not with Gaq or Gas/olf. This interaction occurred in the presence of GTP, but not GDP, in the binding reaction. These findings suggest that Rhes may bind to activated Gai/o to promote inhibition of AC. This, in turn, would result in a net decrease in the ability of Gas/olf-coupled receptors, such as D1, to activate AC. Supported by NCR, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"; PI: Nicolas Bazan and DK073446 (CS).

NS-O12. Tolerance development to hallucinogen-elicited head twitch behavior in mice

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Hallucinogenic drugs with agonist affinity at serotonin 5-HT_{2A} receptors elicit a characteristic head twitch response in mice which has been argued to represent a selective murine model for hallucinogen-like drug effects. In humans, repeated administration of hallucinogens is known to blunt the subjective effects of these drugs, but the consequences of repeated drug exposure on expression of head twitch behavior in the mouse are largely uncharacterized. In these studies, adult male Swiss Webster mice were injected with various doses of the phenethylamine hallucinogen 2,5-dimethoxy-4-iodoamphetamine (DOI) or the tryptamine hallucinogen dipropyltryptamine (DPT) to generate dose-effect relationships for these compounds on head twitch behavior. Behaviorally-equivalent doses of DOI and DPT, or saline vehicle, were then repeatedly administered every 24 or 48 hours, for 5 total injections, and head twitch behavior was quantified. Repeated injections of DOI resulted in a progressively blunted head twitch response, and the development of this tolerance was more rapid when DOI was injected every 24 hours than when it was injected every 48 hours. In contrast, repeated DPT administration did not result in tolerance development, regardless of the inter-injection interval. These findings further underscore the differences between phenethylamine- and tryptamine-based hallucinogens in animal models, and may suggest important distinctions in their drug-receptor interactions. Further pharmacological implications of these data will be discussed. These studies supported by USPHS grants DA020645 and RR020146.

NS-O13. Comparative Genomics and Structure-Function of Projectin in Relation to the Elasticity of Insect Flight Muscles

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The acquisition of flight in pterygote insects is considered a major "stepping stone" in the course of evolution, and a major reason for the success and diversity of insects. One of the critical characteristics of insect flight muscle is their passive and active stiffness. It has been postulated that the stiffness is provided in part by an elastic third filament system, the connecting C-filaments. The projectin protein is one of the two known proteins of the C-filament and as such is proposed to be a major contributor to the IFM's high resting stiffness. One of projectin's regions known as the PEVK domain is a candidate for providing the protein's elasticity. We will present information from our gene sequencing and bioinformatics analysis in the waterbug, *Lethocerus indicus*, in particular the

lack of conserved primary sequence within the PEVK domain. *L. indicus* does not have a genome project, but is a model system in the analysis of insect flight muscles as it possesses very stiff Indirect Flight Muscles (IFM). We are also examining the biophysical and mechanical properties of the PEVK domain in several insects with differing stiffness levels. We will present our progress towards the construction and expression of a series of PEVK-fusion proteins and their analysis by Atomic Force Microscopy (in collaboration with Dr. Wang at NIH). We are also testing the hypothesis that different projectin PEVK isoforms and combinations of such isoforms can confer small differences in muscle stiffness, and ultimately mechanical properties of flight. These experiments are performed on the dragonfly, *Libellula pulchella* as collaboration with Dr. Marden at PennState. The projectin sequence was determined for *L. pulchella*, revealing complex alternative splicing events within the PEVK region. After each animal is tested for mechanical data, the basalar flight muscles are dissected and their RNA extracted. RT-PCR reactions are performed using fluorescently labeled primers and run by capillary electrophoresis. The ratio of the different projectin PEVK splice forms is calculated using fluorescence measurement of peak intensity and statistical analysis. We have evidence for variation between individual *L. pulchella* for the presence and ratio of several PEVK splice forms. We will present ongoing experiments from these approaches and the data analysis to correlate projectin's structure, PEVK mechanical behavior and muscle stiffness.

NS-O14. Stimulation of Histamine H3 Receptors Improves Memory in an Animal Model of Memory Disorders.

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Antagonists of the H3-type histamine receptor possess the ability to alleviate spatial memory deficits produced by MK-801, an antagonist at NMDA-type glutamate receptors that produces brain changes analogous to those found in some human memory disorders. The current study was performed to determine if the H3 agonist, imetit, would exacerbate the detrimental effects of MK-801 on spatial memory. Rats were administered a pretreatment of saline, imetit 3.0 mg/kg or imetit 10 mg/kg 40 minutes prior to memory testing, and received a treatment of saline or MK-801 (0.1 mg/kg) 20 minutes prior to testing. Rats were tested in the radial arm maze apparatus to evaluate memory under varying levels of cognitive load. MK-801 administration produced deficits in all measures of memory, but the 3.0 mg/kg dose of imetit alleviated some of these deficits rather than enhancing them, as predicted. These results suggest that the contribution of H3 receptors to memory function is a complex one, and that agonism of H3 receptors has the capacity to actually improve memory under some neurobiological circumstances.

NS-O15. Galanin expressing leptin receptor neurons and their role in anorexigenic leptin action

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The adipocyte derived hormone leptin acts in the brain to decrease food intake and body weight and its critical role to regulate energy homeostasis is demonstrated by morbid obesity and excessive hyperphagia in humans and rodents lacking leptin or its receptor (LepRb). LepRb expressing neurons are found in several distinct brain sites and can be further sub-categorized by co-expressed neuropeptides, but the contribution of each of these LepRb populations is not well understood. Contrary to initial research recent literature indicated that well studied LepRb neurons in the arcuate nucleus may not mediate major anorexigenic leptin action (van de Wall et al 2008). Here we describe a novel set of LepRb neurons that co-express the neuropeptide galanin - LepRb(Gal) neurons – found in the perifornical area and adjacent ventral dorsomedial hypothalamus (that we collectively term extended perifornical area –exPFA) and a smaller population exist in the brainstem. LepRb and galanin neurons innervate the paraventricular nucleus (PVN), however, the anatomical location of these PVN innervating neurons have not been investigated in detail. We used an adenoviral construct with cre-inducible farnesylated green fluorescent protein expression (Ad-GFPf)(Leshan et al 2009) for specific tracing of LepRb projections from the exPFA (of which about 60% represent LepRb(Gal) neurons). Stereotaxical, unilateral Ad-GFPf injection into the exPFA of LepRbCre mice resulted in GFPf expression confined to LepRb neurons in the exPFA, likely containing LepRb(Gal) neurons. Importantly, we found strong projections from exPFA LepRb neurons into the parvocellular PVN, where neuroendocrine and preautonomic neurons are located that have an important role in the regulation of energy homeostasis and thus suggest that leptin acts on exPFA LepRb neurons to control these PVN outputs. To further test the physiological relevance of leptin action specifically in the exPFA, we injected a single leptin dose (20 or 1pg) into the exPFA (100nl at 10nl/20sec) resulting in a robust reduction of food intake and body weight compared to PBS treated mice ($p < 0.01$). These data support a critical role of exPFA LepRb neurons, likely involving LepRb(Gal) neurons, to mediate anorexigenic leptin action via projections into the parvocellular PVN. Further investigations will be necessary to determine if this action involves the orexigenic neuropeptide galanin as a neurotransmitter. Grants: NIH1 P20 RR02195, AHA053298N to HM.

NS-O16. Atypical antipsychotic treatment is associated with increased complexity of thalamic neuronal dendrites.

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Schizophrenia is a profoundly debilitating neurological disorder with an onset in early adulthood, characterized by psychoses (positive symptoms), blunted affect

(negative symptoms), and reduced attentional and cognitive functions. Recent evidence suggests involvement of the thalamus in some symptoms of schizophrenia: thalamic volumes are reportedly reduced in schizophrenic patients, and neuronal functions associated with thalamic activity - such as attention and EEG synchrony - are disturbed. Whereas typical antipsychotic drugs have limited ability to reverse negative and cognitive symptoms of schizophrenia, newer, "atypical antipsychotics" are reported to have greater efficacy in treating these symptoms. Since alterations in brain volume may represent changes in the amount of dendritic arbor, and in turn network connectivity, we investigated whether treatment with typical or atypical antipsychotic treatment was associated with changes in dendrite morphology within the thalamus and its major efferent, the prefrontal cortex. Rats were treated chronically with the atypical antipsychotic clozapine (10mg/kg/day) or the typical antipsychotic haloperidol (0.1mg/kg/day), and the morphology of neuronal dendrites within the mediodorsal thalamic nucleus and the prefrontal cortex were assessed quantitatively using Golgi-Cox staining. Treatment with clozapine, but not haloperidol, increased the total amount of arbor and dendritic complexity of mediodorsal thalamic neurons, compared to controls. In contrast, neither treatment with clozapine nor haloperidol had any significant effects on dendrite morphology in prefrontal cortical neurons. Since dendritic complexity determines the firing patterns of neurons, these data suggest that the ability of clozapine to improve cognitive abilities may be due to alterations in neuronal network properties mediated at the level of the thalamus. These results implicate thalamic neuronal connectivity in pathology associated with schizophrenia, and outline a possible target for future drug therapies. Supported by NCR, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"; PI: Nicolas Bazan.

NS-O17. Elimination of hyper-reflexia in SCI with modafinil

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Hyper-reflexia occurs after spinal cord injury (SCI) and can be assessed by measuring low frequency-dependent depression of the H-reflex. Previous studies from our labs established that passive exercise of the hindlimbs for 30 days would normalize the H-reflex in the spinal transected adult rat. We also detected changes in the expression of the neuronal gap junction protein connexin 36 (Cx36 mRNA) following transection and after passive exercise. Two labs, Llinas at NYU and Garcia-Rill at UAMS, established that one of the mechanisms of action of the stimulant modafinil is to increase electrical coupling in neurons. This project tested the hypothesis that modafinil administered to transected rats would normalize low frequency-dependent depression of the H-reflex, which is usually decreased by spinal transection. Adult female rats (n=24) were transected at T12 and oral modafinil (4 mg/kg) (n=8), or passive exercise (n=8), treatment begun 7 days after transection, while a control group was treated only with oral vehicle (n=8). The H-reflex was measured 30 days after the beginning of treatment, and the tissue below the transection sampled for changes in Cx36 protein levels. Our results show that oral modafinil or passive exercise for 30 days will normalized the H-reflex in spinal transected adult rats to a similar extent, but vehicle treated rats were hyper-reflexive. The total amount of Cx36 protein in the spinal cord decreased after transection, and there were regional differences in the distribution of Cx36 mRNA levels within the spinal cord. These results suggest that, a) electrical coupling via the neuronal gap junction protein Cx36 is one po-

tential mechanism involved in hyper-reflexia after SCI, b) passive exercise may be normalizing electrical coupling at the level of the spinal cord, and c) the use of the stimulant modafinil is a promising novel therapy for hyper-reflexia and perhaps spasticity after SCI. Supported by NCRN award P20 RR20146.

NS-P1. Docosahexaenoic acid (DHA) modulates survival signaling after focal cerebral ischemia in rats

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The reperfusion phase of brain ischemia-reperfusion triggers the activation of pro-inflammatory, pro-apoptotic protein signaling cascades. Docosahexaenoic acid (DHA) systemically administered attenuates infarct size and enhances recovery after middle cerebral artery occlusion (MCAo) (Belayev et al, Stroke, 2009). We hypothesize that DHA elicits neuroprotection by amplifying the activation of alternative pro-survival cascades thus tipping the overall cellular fate toward survival and recovery. Sprague-Dawley rats (280-300g) underwent middle cerebral artery occlusion for 120mins followed by reperfusion. Animals were randomly assigned to one of three treatment groups:(1)5mg DHA/kg rat body weight in 1.5mL saline(n=5);(2)vehicle:1.5mL saline (n=4); and (3)sham (n=2):mock surgery. Treatment was administered into the femoral vein 60 minutes after suture removal. All rats had a total neurological score of 0 (normal) before ischemia and a high-grade neurological deficit (at least 11 of a maximum 12) 60min after onset of MCA occlusion. Animals were sacrificed after 24 hours via ice cold perfusion with saline. Brain protein extracts from the penumbral region were analyzed by Western blots to compare the relative abundance of total and phosphorylated forms of AKT1 and 14-3-3eta proteins across treatment groups. Stroke resulted in an upregulation of AKT1 in the ipsilateral versus contralateral side of both saline and DHA treated animals by 129% and 187% respectively. DHA treated animals had a 216% increase in the expression of AKT1 relative to saline treated animals. DHA animals also showed an increase in p14-3-3eta on the ipsilateral side versus the contralateral regions (T-Test<0.07) and control animals (T-Test<0.01). Saline animals had decreased levels of p14-3-3 on the ipsilateral versus contralateral side indicating a dysregulation of p14-3-3 signaling in saline treated animals. This data suggests that DHA-mediated neuroprotection triggers the initiation of alternative pro-survival signaling pathways involving AKT1 and 14-3-3eta distinct from the pro-apoptotic cascades after stroke. Supported by NCRN, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"(PI: Nicolas Bazan); NINDS R01 NS046741 (NB) and by a Ruth L. Kirschstein National Research Service Award 1F30AG032841 (TN).

NS-P2. Synaptic activity in cultured primary neurons modifies lipid mediator responses to NMDA excitotoxicity.

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Pharmacologic network disinhibition in cultured primary cortical neurons causes increased synaptic activity and protects cells from a variety of death triggers. Bioactive lipid messengers derived from the polyunsaturated fatty acids (PUFA) arachidonic acid (AA) and docosahexaenoic acid (DHA) may modify cell survival outcomes. We combined automated image analysis of nuclear morphology with high performance liquid chromatography and electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS) to examine correlation between cell survival and lipid messenger profiles. NMDA stimulated lipoxygenase enzyme activity, resulting in the formation of DHA hydroxylation products which may serve as precursors to potent, anti-apoptotic neuroprotectins. Synaptic activity reduced NMDA excitotoxicity and potentiated prostaglandin E2 (PGE2) synthesis. Our approach to mediator lipidomics may provide new insights into the regulation of PUFA-derived mediators by synaptic stimulation. Supported by COBRE, NINDS, and the Ruth L. Kirschstein National Research Service Award.

NS-P3. Up-Regulation of 15-lipoxygenase in Hippocampal Neurons Protects Against NMDA Toxicity

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The aim of our study was to obtain primary cultures of rat hippocampal neurons over-expressing the 15-lipoxygenase (15-LOX-1) open reading frame and also the shRNA in order to produce the enzyme knock-down. The transfected cultures were then examined for their susceptibility to NMDA-induced apoptosis. The hypothesis being tested is that Neuroprotectin D1 (NPD1), a bioactive lipid mediator which is synthesized by 15-LOX-1, is involved in the anti-apoptotic response elicited by NMDA neurotoxicity. Primary hippocampal cultures were maintained in culture for 20 days and then transfected with 15-LOX-1 or shRNA. The cultures were treated with 50 μ M NMDA for 30 min and then incubated for 24 hours. After fixation, the cultures were examined for protein expression (immunocytochemistry) and neuron apoptotic ratio (Hoechst). Our results indicate that 15-LOX-1 transfected cultures displayed significantly reduced apoptosis after NMDA treatment, whereas shRNA transfected cultures were significantly more apoptotic. The 15-LOX-silenced cultures also showed increased sensitivity to NMDA when examined morphologically. Our results support previous data suggesting that bioactive lipids synthesized via the 15-lipoxygenase pathway (such as NPD1) are neuroprotective. Supported by NCRR, COBRE P20 RR016816, Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience; PI: Nicolas Bazan.

NS-P4. The role of broadband sensitivity in the midbrain for auditory temporal processing

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This project uses COBRE support to elucidate mechanisms of complex auditory processing. For example, auditory temporal acuity is critical to acoustic communication in general and speech in particular, as the envelope of speech exhibits complex changes that delineate the temporal boundaries of different speech components. Temporal acuity may be measured using gap detection: the ability to detect a silent time inserted into an ongoing signal. With respect to narrowband or tonal sounds, one cue for gap detection is the spectral “splatter” created by the gap’s interruption of the ongoing sound. It is hypothesized that neurons with broad spectral sensitivity should be more sensitive to gaps due to their increased ability to detect such spectral splatter. For this to be true, however, broad spectral sensitivity must result from the convergence of independent channels, in which stimulation by a single tone does not cause adaptation of the rest of the excitatory band. We tested this hypothesis in phasic neurons in the inferior colliculus (IC) of an awake preparation. The IC is the target of ~8 ascending sources and is one of the first nuclei to show convergence of critical bands from the auditory periphery. Thus, from a circuit point of view, the IC sits at a higher stage of processing than does the visual cortex, where only one synapse may exist between it and the retina (LGN). The function of this convergence in the context of temporal processing is tested by measuring gap detection threshold in single neurons during free field broadcasts. Gaps of varying duration were centered in a 200 ms sinusoid at a frequency within each unit’s excitatory band. Our results show that units with broadband sensitivity are more likely to detect gaps in sinusoids. At the systems level, we show the mechanism for such processing is the ability of broadband sensitive units to function as multi-channel integrators: portions of the broadband are processed independently so that one portion does not elicit adaptation in another, allowing for detection of the splatter. These results are important to therapies for hearing loss, as they reveal the need of prosthetics to restore broad spectral sensitivity (e.g., using cochlear implants) for temporal processing. These data are part of two (R01, NSF) proposals in review and one in preparation (R01). Supported by NCCR, COBRE P20 RR016816, “Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience”; PI: Nicolas Bazan.

NS-P5. Does NPY mediate the behavioral changes produced by anabolic steroids during puberty?

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Anabolic Androgenic Steroids (AAS) are synthetic compounds that resemble the hormone testosterone. Originally, they were used for the treatment of medical conditions. Lately, their abuse has increased alarmingly, especially in adolescents. Puberty is a period of susceptible hormonal changes, and abuse of AAS during this stage may disrupt behavioral patterns. Unfortunately, there is little information on the behavioral and cel-

lular consequences of AAS abuse during puberty. AAS can affect neuropeptides leading to modulation of behavior. Neuropeptide Y (NPY), the most abundant in the CNS, is related to cognition, anxiety, feeding, and sexual behavior. Previously, our laboratory showed an increase in NPY levels in the ventromedial nucleus of the hypothalamus (VMN) in male pubertal rats after AAS treatment, while pubertal females showed a decrease in NPY levels in the amygdala (AMY). We aimed to assess if chronic exposure to 17 α -methyltestosterone (17 α -meT; 7.5 mg/kg) will alter behaviors controlled by these brain regions (i.e. sexual behavior, anxiety and emotional memory) in male and female pubertal rats. Motivational components of the sexual behavior were the most affected. Decreases in the latency to the first mount and first ejaculation were detected. In addition, an increase in multiple ejaculations was also observed after AAS exposure, while copulatory behavior was not affected. In female pubertal rats, the Inhibitory Avoidance Task (IAT) and the Elevated Plus Maze (EPM) were used to assess the effects of 17 α -meT on emotional memory and generalized anxiety respectively, both amygdala-dependant behaviors. In the IAT we observed a tendency to impair emotional memory, while no effect was observed in the EPM after exposure. Body weight and food intake were also measured. AAS-treated males showed a significant increase in body weight with no changes in food intake. In contrast, there was a significant increase in body weight and food intake in females. Animals were sacrificed and brain punches were obtained for further studies of NPY levels using Real Time-PCR. In male rats, 17 α -meT decreased mRNA levels for NPY, receptors NPY Y1, NPY Y2, NPY Y5, and the androgen receptor (AR) in the VMN. In females, AAS treatment did not affect mRNA transcripts in the amygdala. This study suggests that NPY might be an important cellular substrate underlying the AAS-induced behavioral changes. Support-RCMI (G12RR03051), NIH-NCRR (P20RR016470), EARDA-NIH (G11HD046326).

NS-P6. Psychosocial adversity in childhood and physical health in adulthood: Exploring connections and potential mechanisms

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 Jennifer Reinovsky, Furman University
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A growing body of evidence suggests that harsh, unsupportive family environments or substantial economic difficulties during childhood are associated with an increased risk of poor medical outcomes in adulthood. That these associations are above and beyond the established effects of adult psychosocial processes on physical health indicates the unique nature of experiencing significant stressors in childhood. The goal of my current line of research is to better understand the link between childhood adversity and adult physical health, as well as to explore possible explanations for this link. One research project involved a quantitative synthesis of the child abuse and adult health literature to see whether experiencing child abuse increases the risk of developing a physical illness in adulthood. Our results revealed that the magnitude of the association between child abuse and physical health was as large as the association between child abuse and mental health, suggesting that a harsh early environment can leave lingering effects on the body as well as the mind. A second study addressed a potential mechanism to explain the connection between stress in childhood and poor physical health in adult-

hood: altered stress reactivity. Intense or prolonged stress during childhood may alter the way the body responds to future stressors and increase risk for negative health outcomes. In order to explore this possibility, a group of adults from the Greenville community reported their childhood socioeconomic status (whether their parents rented or owned their residence when they were born). Participants then completed an evaluative interview and serial subtraction task in the laboratory while their blood pressure and heart rate were measured. Participants whose families rented at the time they were born displayed a blunted cardiovascular response to the laboratory stressor compared to participants whose families owned their residences when they were born. This difference persisted after controlling for the participant's current socioeconomic status. These findings are consistent with the idea that psychosocial adversity during childhood negatively affects the body's stress response systems. A maladaptive response to stress can contribute to future disease risk.

NS-P7. Testing if Cryptochrome is the Primary Photoreceptor Involved in Resetting the Circadian Clock in *Chlamydomonas reinhardtii*

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Cryptochrome is a strong candidate for the primary photoreceptor that perceives light as an external time cue to reset the circadian clock in the unicellular green alga *Chlamydomonas reinhardtii*. To test whether cryptochrome performs this task, the strain CC48 was transformed using an RNA interference construct designed to silence the production of cryptochrome. The transformants were then screened for reduced cryptochrome using western blot analysis. Several strains were found to have cryptochrome production reduced to various degrees. CC48 and one strain with reduced cryptochrome, RNAi #16, were then grown in a strict 12-hour light/12-hour dark cycle to synchronize the cultures to a known time frame. At specific times in the dark phase, samples from these cultures were treated with light pulses of varying light intensities, which would cause a phase shift in their rhythms if the photoreceptor responsible for perceiving these time cues were present in the cells. After the light treatment, the samples were placed into a machine, which monitored their circadian rhythm of phototaxis, or swimming towards light. The data collected by the machine were visualized and analyzed using a Mathematica program. The data analysis was used to determine the phase shift of each sample compared to the control without light treatment. The phase shifts of the wild-type strain, CC48, were compared to those of RNAi #16 to determine if the reduced cryptochrome in RNAi #16 reduced its ability to reset its circadian clock. At the current light intensities, there appeared to be no reduction in the ability of RNAi #16 to reset its clock, but several tests need to be performed with light pulses at a lower intensity and at a specific wavelength.

NS-P8. Identifying Cellular Pathways Modulated by Drosophila Palmitoyl Protein Thioesterase 1 Function

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Infantile-onset Neuronal Ceroid Lipofuscinosis (INCL) is a severe pediatric neurodegenerative disorder produced by mutations in the gene encoding palmitoyl-protein thioesterase 1 (Ppt1). This enzyme is responsible for the removal of a palmitate post-translational modification from an unknown set of substrate proteins. To better understand the function of Ppt1 in neurons, we performed a dominant loss-of-function genetic modifier screen in *Drosophila* using a previously characterized Ppt1 over-expression system. The modifiers identified in our screen implicate Ppt1 in cellular trafficking and the modulation of important cellular pathways, such as Bone Morphogenetic Protein signaling. We further support the findings of our screen by demonstrating that Ppt1 mutant cells have defects in endocytosis. Finally, we show that the *Drosophila* Ppt1 homolog is localized to the acidic endo-lysosomal compartment *in vivo*. This work points to alterations in cellular trafficking and specific signaling pathways as major contributors to the INCL disease process.

NS-P9. Mechanism of Acetylcholinesterase's Neurotrophic Action on DRG Neurons of Rat

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The cholinergic neurotransmitter system has been the focus of extensive research with regards to its role in neurotransmission. Especially acetylcholinesterase (AChE), the enzyme which terminates the action of neurotransmitter acetylcholine has received much attention as some of the inhibitors of AChE are recognized as potent pesticides and nerve gas agents. However, emerging evidence indicates that this enzyme as well as cholinergic receptors play significant roles in growth and neuromodulation in addition to neurotransmission. Several studies including ours show that AChE promotes neurite growth and neuron survival in a dose dependent manner in invertebrate as well as vertebrate neurons. AChE's neurotrophic action appears to be non-catalytic. To understand the underlying mechanism, we have chosen a dissociated cell culture model of dorsal root ganglion (DRG) neurons of rat and exposed them to AChE isolated from fetal bovine serum. In addition to measuring neurite growth, we performed acetylcholinesterase binding experiments and microarray analysis of gene expression profiles in control and AChE-exposed DRG neurons. AChE exposure significantly increased neurite length and neuron survival dose dependently. Further fluorescence beads coated with AChE showed binding to DRG neurons. Among the 1200 gene probes in the Affymetrix rat neuron chip, the expression profiles of 22 genes were significantly altered in DRG neurons as a result of their exposure to AChE in all the three repeats of the experiment. Significant among them were receptors for epidermal growth factor (EGF) and ciliary neurotrophic factor (CNTF). To investigate how AChE may influence the expression of receptors for EGF and CNTF, experiments are underway in our laboratory to isolate and characterize the molecule in DRG neurons that binds to AChE. These results add to

the growing body of evidence which shows significant, non-neurotransmission related roles for cholinergic components in neuron survival and growth. These studies are supported by NIH Grant Number P20 RR-16460 from the IDeA Networks of Biomedical Research Excellence (INBRE) Program of the National Center for Research Resources, used equipment provided by funds from NIH/NIDA and NSF/EPSCoR funds and also ABI funds for animal maintenance to M. Srivatsan.

NS-P10. In vivo measurements of visual motion selectivity in the midbrain using 2-Photon imaging

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Through COBRE funding this project is developing a novel in vivo method to examine neuronal plasticity in sensory processing. The developing brain has extensive capacity to learn new sensory stimuli, a trait critical to acquiring communication skills such as speech and writing. At the systems level, data showing sensory development and plasticity are often methodologically limited, as either single unit or gross physiological assays (e.g., fMRI) are unable to measure adjacent circuitry or single cell responses, respectively. This project, however, uses 2-photon microscopy in vivo to simultaneously define the development of both the single cell and ensemble circuitry involved in sensory processing. In particular, neurons in the frog optic tectum (superior colliculus), are selective to the direction of movement of visual objects. Our goal is to measure how such circuitry acquires and changes directional selectivity during development. Using a membrane-permeate calcium indicator dye (Oregon Green 488 BAPTA-1 AM ester) injected into the live *Xenopus* tectum, 2-photon microscopy is able to visualize the responses of multiple neurons and glia (single cell resolution) to visual stimuli presented directly to the eye via an optic fiber bundle. This method resolves both excitation and inhibition, as changes in fluorescence indicate changes in internal Ca²⁺, resulting from increases or decreases in membrane potential, respectively. Responses are monitored using 820-910 nm wavelengths. Presently, the project is characterizing motion detection of a single developmental stage with initial results showing that such circuitry is complex, as adjacent neurons interestingly can exhibit opposite directional selectivity. These data are part of two (R01, NSF) proposals currently in review. Supported by NCRR, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"; PI: Nicolas Bazan.

NS-P11. Effects of nicotine and cigarette smoking on the responses of cholinergic cells within the reticular activating system responses

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In the rat the vertex-recorded P13 and N40 midlatency auditory evoked potentials occur

~13 and 40 msec after appropriate stimulation. These waveforms have been localized to the reticular activating system (the pedunculo-pontine nucleus) and the hippocampus respectively. These responses have been used as measures of arousal and their habituation plays a role in sensory gating. The results of our experiments show that both of these event related potentials are affected by agonists and antagonists of nicotinic acetylcholine receptors and habituate rapidly to repetitive stimuli. Specifically we have shown that: 1) Nicotine injections reduce the amplitude of these potentials in a dose-dependent but transient manner. 2) These effects were blocked by injection of nicotinic receptor antagonist (mecamylamine) and duplicated by injection of a nicotinic agonist (DMPP) into the pedunculo-pontine nucleus (PPN). 3) Exposure to cigarette smoke produced similar effects and decreased habituation of these responses. These effects were dose-dependent, but lasted only a few minutes. 4) Pups born to dams exposed to cigarette smoke during pregnancy showed reduction in habituation of the P13 response that persisted throughout puberty. This result suggests that in utero exposure to the constituents of cigarette smoke resulted in long-lasting alterations in stimulus processing associated with sensory gating. This deficit may contribute to attentional and cognitive defects as well as disturbances in perception that could partially explain the effects of maternal smoking. 5) In vitro studies showed changes in the intrinsic electrical properties of cholinergic PPN cells consistent with increases in excitability. These results could partially explain the differences seen in individuals whose parents smoked during pregnancy, especially in terms of their arousal level and increased propensity for anxiety disorders. Together, these results provide important insights into the mechanisms responsible for some of the responses to smoking and effects of maternal smoking. Supported by: NIH NCCR grants P20 INBRE RR-16460, P20 COBRE RR-020146 and the: Arkansas Biosciences Institute.

NS-P12. Molecular Mechanisms of Transcriptional Regulation by KChIP3/DREAM/Calsenilin

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Potassium Channel Interacting Proteins (KChIPs) are members of a family of calcium binding proteins that interact with potassium (K⁺) channels and can also act as transcription factors. KChIP3 is also known as calsenilin and as the transcription factor, Downstream Regulatory Element Antagonist Modulator (DREAM), which is known to regulate transcription of a number of genes. KChIP3 is highly expressed in the hippocampus, an area of the brain important for learning and memory. We evaluated the role of KChIP3 in a hippocampus-dependent memory task, contextual fear conditioning. Male KChIP3 KO mice showed significantly enhanced memory after training as measured by percent freezing. We found that nuclear KChIP3 expression was increased 6 hours after the fear conditioning training paradigm in WT animals compared to animals exposed to the context but not trained, suggesting a nuclear role for KChIP3 after training. Furthermore, prodynorphin mRNA expression was significantly decreased 6 hours after fear conditioning training in WT but not in KO animals. These data suggest a role for regulation of gene expression by KChIP3/DREAM/calsenilin in consolidation of contextual fear conditioning memories. Little is known, however, about the molecular mechanisms of transcriptional regulation by KChIP3/DREAM/calsenilin. In this study, we investigate the posttranslational modifications of KChIP3 that are necessary for translocation to the nucleus and DNA binding. Previous studies in neuroblastoma cells suggest that an increase in intracellular Ca²⁺ is necessary for translocation of KChIP3/DREAM/

calsenilin to the nucleus (Zaidi et al, J Neurochem. 89:593-601; 2004). We will investigate this mechanism in neurons. In addition, we show that phosphorylation of KChIP3/DREAM/calsenilin by PKA is necessary for DNA binding in vitro and inhibition of ERK/MAPK reduces KChIP3/DNA binding in the slice preparation. These studies will lead to an enhanced understanding of the molecular mechanisms of transcriptional regulation by KChIP3/DREAM/calsenilin. Supported by NCRR, COBRE P20 RR016816, "Mentoring Neuroscience in Louisiana: A Biomedical Program to Enhance Neuroscience"; PI: Nicolas Bazan

NS-P13. Chronic restraint stress down-regulates Freud-1 but not NUDR in the rat prefrontal cortex

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Freud-1/CC2D1A; five prime repressor element under dual repression/coiled-coil C2 domain 1A and NUDR/Deaf-1 (nuclear deformed epidermal autoregulatory factor) are two important regulators of the 5HT1A receptor. In this study we show that after 21 days of chronic restraint stress significant reductions in both Freud-1 mRNA and protein were observed in the prefrontal cortex (PFC) (36.8% and 32% respectively). On the other hand levels of NUDR protein and mRNA did not change significantly. Consistent with reduced Freud-1 protein, 5HT1A receptor mRNA levels were equally upregulated in the PFC, while protein levels declined, suggesting postranscriptional receptor down-regulation. The data suggest that restraint stress produces distinct alterations in the serotonin system in the PFC.

NS-P14. Effects of maternal cigarette smoke exposure and methamphetamine challenge on brain monoamine concentrations in rat pups

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Maternal smoking is correlated with several cognitive and behavioral disorders in children including learning disabilities, attention deficit disorders and higher prevalence of drug use and addiction. The causes of these disorders are not known, however, it is known that nicotine readily crosses the placenta. Because there are many regions of the brain that contain nicotine sensitive cells, in utero exposure to nicotine from cigarette smoke is likely to affect prenatal brain development. The purpose of these studies was to determine the effects of cigarette smoke on re-

gional neurotransmitter concentrations in the brains of pups whose mothers were exposed during the 3rd week of pregnancy and to examine the effects of subsequent methamphetamine challenge on these concentrations. For all experiments, pregnant rats were exposed to cigarette smoke (~385 ml of smoke from 1R3F cigarette) 3 times a day beginning on gestational day 14 until parturition. Other dams were treated identically but not exposed to cigarette smoke. Brains of exposed and unexposed pups were harvested and the caudate nucleus, frontal cortex, hippocampus, hypothalamus, cerebellum and brainstem removed on postnatal days 20, 30, 45 and 60. Tissues were immediately frozen in liq N₂ and stored at -80° C. Concentrations of dopamine, 3, 4-dihydroxyphenylacetic acid, 5-HT, 5-hydroxyindoleacetic acid and homovanillic acid in each tissue were measured using HPLC/EC. For METH challenge experiments, on PNDs 30 or 60 pups were injected with METH (5 mg/kg, 3 times within 12 hr). Animals were sacrificed 48 hr after injections, and brain regions were harvested. In utero exposure to the constituents of cigarette smoke was associated with regional and temporal changes in monoamine concentrations. Animals challenged with METH showed no significant changes in monoamine concentrations at PND 30, but, at PND 60 significant changes were observed in both DA and 5-HT concentration in striatum when animals were challenged with METH. In frontal cortex, no significant changes were associated with in utero cigarette smoke in any time points. However, METH alone produced significant changes in DA concentration. These data show that in utero exposure to the constituents of cigarette smoke produces changes in monoaminergic systems and that Supported by: NIH NCRR grant P20 INBRE RR-16460, and the Arkansas Biosciences Institute and the National Center for Toxicologic

NS-P15. Adherence is Good for You: Uncovering the Mechanisms behind the Healthy-Adherer Effect

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Patient nonadherence to medical treatment is a widespread problem that significantly detracts from the likelihood of a successful treatment. Those who demonstrate good adherence to active drugs have half the mortality risk compared to poor adherence groups. Surprisingly, those who show good adherence to a placebo also exhibit half the mortality risk of poor adherers (Simpson et al., 2006). This phenomenon, known as the healthy adherer effect, is attributed to the fact that good adherers are also more likely to engage in other beneficial health behaviors, such as exercising and not smoking. Adherence is not manipulated in most clinical trials, however, so alternative explanations cannot be ruled out. For example, good adherers may be higher in conscientiousness, a personality trait that has been linked to better health. With this in mind, this study will manipulate adherence by recruiting adults age 30-65 for a purported study of a memory-enhancing drug that is actually a placebo. Participants will be asked to take the drug four times a day for two weeks and will be randomized into one of two conditions: low adherence in which they will receive basic dosage instructions and high adherence in which they will receive detailed instructions and aids to increase their adherence. Both groups will attend two lab sessions in which short-term and working memory will be assessed. In order to avoid self-report and recall biases, adherence will be tracked using a MEMS cap that records when the pill bottle is opened. We hypothesize that those in the high adherence group will demonstrate a larger placebo effect compared to the

low adherence group, as evidenced by better memory scores. This will lend evidence to the assumption that the adherence behavior itself, rather than personality traits or other health behaviors, is the mechanism behind the healthy adherer effect. Data collection is currently under way.

NS-P16. Neurolipidomics Analysis in Animal Behavioral Studies

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Recognition of the uniqueness and complexity of cellular lipids and their interacting moieties in the nervous system empowers the development of a new technology called neurolipidomics which provides the system level analysis of lipid homeostasis in the brain including their diverse structural and biological functions. To date it is becoming more evident that the lipid environment including the content of polyunsaturated fatty acids (PUFAs) in neuronal membranes may have a significant impact on behavior. Moreover, there is considerable evidence that the acceleration of cognitive function can be controlled by PUFAs dietary supplementation. In addition, studies suggest that intensive plasticity-engaging training programs significantly improve memory and learning. Despite these findings, the information about fatty acids content in the brain after training exercises is still missing. Therefore, we propose that animal studies must include a methodical examination of fatty acids composition in neuronal tissue before and after physical exercises. For this aim, 8-week old male C57BL/6 mice were divided into two groups. Running mice were allowed to experience voluntary exercise with a running wheel in their homecages for 30 days. Sedentary mice also remained in their homecages for 30 days, but with no access to a running wheel. Voluntary training exercise was followed by the behavioral test using the elevated plus-maze to assess possible differences in anxiety-related behaviors between the groups. The results obtained showed that active animals have lower levels of anxiety and engage faster in the exploration of the maze than sedentary animals. After completing the behavioral test, both groups of animals were used for profiling of fatty acids pattern in the hippocampus and cortex by GC-MS. The data revealed a noticeable increase in the content of PUFAs - omega-3 and omega-6 in the hippocampus while their content remained unchanged in the cortex. Overall, the present study demonstrates the positive effect of physical activity on emotional state and provides a biochemical basis for understanding lipid complex metabolic network. We expect that further exploiting of this complexity by different academic disciplines such as biology, biochemistry, physiology, chemistry, and mathematics will bring substantial insights into neuronal plasticity thus contributing significantly to translational research and drug development.

NS-P17. Maturation of MNTB neurons occurs in synchrony with the growth of the presynaptic calyx of Held

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Whole cell recordings and calcium imaging experiments were performed in mouse brain slices beginning at embryonic day 17 (E17) through postnatal day (P)14 to study the biophysical maturation of principal neurons of the medial nucleus of the trapezoid body (MNTB) and the relation of this postsynaptic maturation to the growth of the presynaptic calyx of Held. Analysis of spontaneous synaptic events indicated that MNTB neurons were innervated by glutamatergic and GABAergic inputs at E17, when MNTB neurons are first discernable in an organized nucleus. Excitatory postsynaptic currents (EPSCs) and intracellular calcium increases were evoked by stimulating the contralateral cochlear nucleus, indicating that neurons in the cochlear nucleus, likely the calyx-forming globular bushy cells, were capable of propagating action potentials (APs) at these ages. Although EPSC amplitudes were small prior to calyx growth (E17-P1), high input resistance and a depolarized resting membrane potential (RMP) allowed spontaneous and stimulus-evoked synaptic events to drive APs in MNTB neurons before calyces began to grow at P2. We previously established that the majority of MNTB neurons are innervated by a single immature calyx of Held at P4. Here, we found that most MNTB neurons were innervated by multiple inputs from E17-P2. EPSC amplitudes of convergent inputs were similar in amplitude prior to calyx growth, but larger terminals began to emerge at P2 until a single, dominant calyx was formed at P4, suggesting that the smaller inputs may act competitively to determine which terminal will grow into a calyx. Biophysical properties of MNTB neurons begin maturing at embryonic ages and reach adult values either before or during calyx growth. Importantly, the transition to more hyperpolarized RMP and a decrease in input resistance lead to an increase in current thresholds for AP generation at P2. Together, these data indicate that the presynaptic calyx of Held and the postsynaptic MNTB neuron mature in synchrony and suggest that the decrease in excitability of MNTB neurons that occurs just as calyces begin to grow could provide a mechanism that selectively permits larger inputs to grow and eventually form calyces of Held.

NS-P18. Monomeric laforin is likely the biologically relevant form in Lafora Disease

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Lafora Disease (LD) is an autosomal recessive epileptic disorder, which causes rapid neurological deterioration and the accumulation of insoluble carbohydrates called Lafora bodies (LBs). LD patients die within 10 years of experiencing an initial epileptic episode during their second decade of life. LD is caused by mutations in either the gene encoding laforin or malin. Malin is an E3 ubiquitin ligase that ubiquitinates laforin and other proteins involved in glycogen metabolism. Laforin contains a dual specificity phosphatase domain and a carbohydrate-binding module. Laforin is capable of removing phosphate from phosphoglucans and is hypothesized to function in removing phosphate from glycogen. A previous study suggested that laforin is only active as a dimer, which is very rare for cytosolic phosphatases. This report prompted us to probe

the native form of laforin and investigate the functional and physiological relevance of the monomer and dimer. We purified human laforin, performed size exclusion chromatography, and subjected the monomer and dimer fractions to coomassie staining, mass spectrophotometry, immunoblotting, and phosphatase activity measurements. Our results show that laforin prevalently exists as a monomer. When normalized to total laforin content, the ability of laforin dimer to remove phosphate from pNPP was higher than the monomer. More importantly however, monomer and dimer possessed equal activity in removing phosphate from a physiologically relevant substrate. Our data suggests that laforin monomer is the most biologically relevant form given its greater abundance and equal phosphatase activity compared to the dimer form. Thus, detailed study of the laforin monomer is needed for better understanding of the genesis and pathophysiology of LD.

Regenerative Medicine

RM-O1. Altered versican processing in mouse models of ADAMTS5 deficiency leads to severely hypertrophic semi-lunar valves

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The proteoglycan versican is essential for endocardial cushion formation, the precursors of adult valves. However the cleavage of versican by the "A Disintegrin and Metalloproteinase with ThromboSpondin motifs (ADAMTS) family of ECM proteinases has not been experimentally examined. ADAMTS5 efficiently cleaves versican and is expressed in the endocardial and mesenchymal cells of the hinge region during valve maturation. To test the hypothesis that versican cleavage is critical for valve development, gene-targeted mouse models deficient in ADAMTS5, (*Adamts5LacZ/LacZ*) were investigated. Our unpublished findings determined that the pulmonary valves (PA) of *Adamts5LacZ/LacZ* mice were grossly enlarged at E17.5 compared to wild type littermates. Three-dimensional reconstructions of both *Adamts5LacZ/LacZ* and wild type (WT) littermates at E17.5 were generated. Volumetric measurements from these reconstructions showed that there was a 4.09 fold total increase in the pulmonary valves of the *Adamts5LacZ/LacZ* mouse compared to WT littermates. Independent analysis of leaflets revealed a 6 fold increase in volume of the left PA leaflet while the anterior and right leaflets each had approximately 3 fold increases in volume compared to WT. The aortic valves showed a 1.60 fold increase in the *Adamts5LacZ/LacZ* homozygotes compared to the WT littermates. The phenotypic findings correlate with a dramatic reduction in versican cleavage from E14.5 to E17.5 in the *Adamts5LacZ/LacZ* compared to WT. Semi-quantitative fluorescent immunohistochemistry obtained by analysis of digital confocal images revealed a significant reduction in the amount of cleaved versican in the *Adamts5LacZ/LacZ* hearts compared to WT. This was complimented by a statistically significant increase in intact versican in the *Adamts5LacZ/LacZ* pulmonary valves when compared to WT tissue. These data suggest that ADAMTS5 cleavage of versican is important for development of the semi-lunar valves of the aorta and pulmonary arteries.

RM-O2. Jarid2 is Differentially Regulated by Nkx2.5 in the Second Heart Field During Outflow Tract Morphogenesis

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Congenital defects of outflow tract (OFT) formation affecting development of the aorta and pulmonary artery comprise a significant proportion of congenital heart disease (CHD). Of the 9/1000 of the human population diagnosed in the first year of life to have CHD, approximately one third have OFT defects. An additional estimated 13/1000 infants are born with bicuspid aortic valve, an OFT malformation most often silent in the first year of life, but often significant in adult life. Nkx2.5, a transcription factor implicated in human congenital heart disease, is required for regulation of second heart field (SHF) progenitors contributing to outflow tract (OFT). Using an innovative SHF-specific expression array analysis, we have identified a set of genes expressed in SHF containing pharyngeal arch tissue whose transcriptional regulation is dependent on Nkx2.5, and whose cognate regulatory regions may directly bind Nkx2.5 through specific binding sites. Jarid2, which has been implicated in OFT morphogenesis, was found to be augmented in SHF mesoderm and related cell populations of Nkx2.5 deficient embryos. Chromatin immunoprecipitation analysis showed that Nkx2.5 interacts with consensus binding sites in Jarid2 regulatory regions in pharyngeal arch cells. Given the role of Jarid2 as a regulator of early cardiac proliferation, these data highlight Jarid2 as one of several potential mediators of the critical role played by Nkx2.5 during OFT morphogenesis.

RM-O3. Loss of FoxO1 in endothelial cells causes decreased cardiac myocyte proliferation during heart development

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FoxO1 is a forkhead/winged helix transcription factor involved in proliferation, apoptosis, and cell cycle regulation during cardiovascular development. Myocyte-specific transgenic expression of FoxO1 during heart development causes severe myocardial defects and embryonic lethality at embryonic day 10.5 (E10.5). FoxO1 null mice also die early during embryogenesis due to defective vascular development. However, loss of FoxO1 in cardiac myocyte-specific lineages does not result in an obvious embryonic or neonatal phenotype and the mice are viable. Thus, while FoxO1 can influence growth of the myocardium, it is not required during heart development. In endothelial cells, FoxO1 regulates endothelial growth through transcriptional regulation of genes that establish a balance between cellular proliferation and apoptosis. Since endothelial cells can influence cardiac myocyte proliferation via cell signaling between the myocardium and endocardium, our aim was to investigate if loss of FoxO1 in endothelial cells affects pro-

liferation and/or apoptosis of cardiac myocytes during heart development. Endothelial-specific FoxO1 knockout mice were generated by crossing mice with a conditional allele of FoxO1 (FoxO1 flox) with a Tie2-Cre line. Tie2-Cre⁺; FoxO1 flox/flox mutant embryos were examined at E9.5 and E10.5. Mutant embryos were present in expected Mendelian ratios and were easily distinguished from littermates by the absence of large blood vessels in the yolk sac as well as gross morphological defects. Embryonic lethality was evident at E10.5 and existing mutant embryos displayed overall growth retardation and signs of cardiovascular failure, including pericardial edema, dilated, blood-filled vessels with localized hemorrhage, and thin, abnormal myocardium. Cardiac myocyte proliferation was decreased by 80% in mutant embryos at E9.75 versus control littermates. Current studies include analysis of proliferation and apoptosis in endothelial cells compared to cardiac myocytes as well as examination of signaling molecules related to endothelial/ myocardial interactions that regulate cardiac myocyte proliferation in mutant embryos. The phenotype of the Tie2-Cre⁺; FoxO1 flox/flox mutant embryos demonstrates that FoxO1 is required in the endocardium to regulate proper formation of the myocardium during heart development and further experiments are required to dissect out the molecular mechanisms involved.

RM-O4. Reduced versican cleavage due to Adamts9 haploinsufficiency is associated with cardiac and aortic anomalies

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Here, we demonstrate that ADAMTS9, a highly conserved versican-degrading protease, is required for correct cardiovascular development and adult homeostasis. Analysis of Adamts9⁺/LacZ heterozygous adult mice revealed anomalies in the aortic wall, valvulosis and valve leaflets. Abnormal myocardial projections and 'spongy' myocardium consistent with non-compaction of the left ventricle were also found in Adamts9⁺/LacZ mice. During development, Adamts9 was expressed in derivatives of the Anterior Heart Field (AHF) vascular smooth muscle cells (VSMC) in the arterial wall and mesenchymal cells of the valves, but expression also continued in the adult heart and ascending aorta. Thus, the adult cardiovascular anomalies found in Adamts9⁺/LacZ hearts could result from subtle developmental alterations in ECM remodeling or defects in adult homeostasis. The valvular and aortic anomalies of Adamts9⁺/LacZ hearts were associated with accumulation of versican and a decrease in cleaved versican relative to WT littermates. Chondrogenic nodules in the valvulosis showed enhanced TGFβ signaling. These data suggest a potentially important role for ADAMTS9 cleavage of versican in both development and allostasis of cardiovascular ECM and identify ADAMTS9 as a potential candidate gene for congenital cardiac anomalies. Mouse models of ADAMTS9 deficiency may be useful to study myxomatous valve degeneration.

RM-O5. SPARC knock out mice have Irregular Collagen Morphology within the Periodontal Ligament

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The periodontal ligament (PDL) is vital to maintaining oral health because the PDL fibers anchor each tooth into the surrounding bone. The PDL also serves to absorb shock from the forces of mastication to prevent bone resorption. Cell types within the PDL include fibroblasts, endothelial cells, and neuronal cells. However, fibroblasts are the main cell type and function to produce collagen and other components that comprise the extracellular matrix (ECM). The ECM of the PDL, composed of fibrillar collagens I and III, undergoes high rates of collagen turnover mediated by resident fibroblasts. SPARC, a matricellular protein, associates with collagens I and III and is implicated as a mediator of collagen turnover and phagocytosis. We have studied the role of SPARC in the PDL using a SPARC knock out murine model and human PDL fibroblast cells. Preliminary results suggest the SPARC knock out mice have decreased numbers of fibroblasts and increased disorganization of existing fibers within the PDL. The phenotype of the SPARC knock out model is more severe as the mice age. Our model has shown that SPARC is essential for the organization of collagen within the PDL.

RM-O6. Second Heart Field Regulation of Nkx2.5, a Critical Gene in Outflow Tract Congenital Heart Disease

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Congenital heart disease (CHD) spans a wide range of structural and functional abnormalities in early heart development, and approximately 35,000 children are born each year with some form of CHD. One-third of these cases are due to malformations of the outflow tract (OFT), which gives rise to both the pulmonary artery and the aorta. The OFT and right ventricular (RV) myocardium (muscular heart tissue) is derived from the second heart field (SHF), a spatiotemporally distinct source of cardiac progenitor cells originating outside the primary heart tube. The cardiac transcription factor gene Nkx2.5 is an important regulator of the SHF program: loss of Nkx2.5 function results in severe OFT and RV hypoplasia, while hypomorphic Nkx2.5 alleles are linked to OFT malformations such as double outlet right ventricle or Tetralogy of Fallot in mouse models and in humans. The Nkx2.5 gene operates within a complex network of upstream regulators and downstream target genes, many of which are also implicated in OFT CHD. As part of our effort to characterize upstream modifiers of Nkx2.5-related OFT CHD, we are characterizing species-specific SHF enhancers from the chick and mouse Nkx2.5 genes. These enhancers appear to represent two major classes of archetypal regulatory regions conserved through various vertebrate species. Comparative analysis of common versus divergent TF binding motifs and high throughput knockout experiments will provide insight into the critical transcriptional events governing SHF specific expression of Nkx2.5 and their effect on OFT morphogenesis.

PANEL BIOGRAPHIES

W. Scott Argraves

Dr. Scott Argraves has served as director of shared resources cores at MUSC since 1994. The services offered through his cores have included technologies ranging from protein sequencing and surface plasmon resonance to DNA microarray and multiplex bead array expression profiling and bioinformatics. He currently serves as the director for several integrated cores supported from both NIH grants and institutional funds. These cores include the Proteogenomics Core for the NIH/NCRR-P20 RR016434 funded grant, "South Carolina COBRE for Developmentally Based Cardiovascular Diseases" (2006-2011), the Proteogenomics Core supported by funds through the Provost's office since 1996, and the Bioinformatics Core of the NIH/NCRR-P20 RR16461 funded grant "South Carolina IDeA Networks of Biomedical Research Excellence."

Kathleen T. Brady

Dr. Kathleen T. Brady is a board-certified psychiatrist specializing in addiction psychiatry. As a Professor of Psychiatry at the Medical University of South Carolina, Dr. Brady is Director of the Clinical Neuroscience Division, Director of the Southern Consortium of NIDA's Clinical Trials Network, Director of MUSC's Clinical and Translational Science Award and Associate Dean for Clinical and Translational Research. Dr. Brady received her PhD in Pharmacology from Virginia Commonwealth University and her MD degree from the Medical University of South Carolina, where she completed a residency in psychiatry, served as Chief Resident, and completed an Addiction Psychiatry fellowship. Her research interests are in the areas of drug and alcohol abuse/addiction and comorbid conditions such as posttraumatic stress disorder and other anxiety disorders. She has served as Principal Investigator, Co-Principal Investigator, and Mentor on numerous research projects. In addition, she has received awards for her research, teaching, and clinical work, and has been listed in "Best Doctors in America" since 1998. Dr. Brady has been very active in organizations addressing the concerns of psychiatry and addictions. She is Past President of the Association for Medical Education and Research in Substance Abuse and Past President of the American Academy of Addiction Psychiatry. She has served on the Scientific Advisory Council of the National Institute of Drug Abuse and the Committee on Community Based Treatment of the Institute of Medicine for the National Academy of Science, as well as the Board of Directors of the College of Problems of Drug Dependence.

Lawrence Cornett

Dr. Lawrence Cornett earned a BS in biology from the University of California, Riverside, and a PhD in physiology from the University of California, Davis, and he was a post-doctoral fellow in reproductive endocrinology and cardiovascular physiology at the University of California, San Francisco. He joined the University of Arkansas for Medical Sciences faculty in 1980 and is currently a Professor in the Department of Physiology and Biophysics, the Executive Associate Dean for Research in the College of Medicine, and the Vice Chancellor for Research for the campus. His research interests include the role of β_2 -adrenergic receptors in mediating airway responsiveness in asthma and hormonal regulation of stress responses in avian species. In addition, Dr. Cornett is the

Director of the Arkansas INBRE and an Associate Director of the Arkansas Center for Clinical and Translational Research. Among his many honors, Dr. Cornett has received a fellowship from the NIH Fogarty Center and a Research Career Enhancement Award from the American Physiological Society.

Anthony DePass

Anthony DePass is the Associate Dean for Research and Associate Professor of Biology at the Brooklyn campus of Long Island University. He has extensive experience in the administration and evaluation of programs aimed at faculty and student development. Additionally, he is the Principal Investigator and Director of Long Island University MBRS SCORE program and MARC (T36) award to the American Society for Cell Biology, where he currently serves as the Chairman for the Minority Affairs Committee. Under his leadership, the ASCB MAC runs development programs targeting individuals from the undergraduate to the faculty levels, where several hundred participants representing over 140 institutions have been directly impacted. One activity under the ASCB MARC program is the annual conference on Interventions that Encourage Minorities to Pursue Research Careers. Dr. DePass has served as Chair and Co-Chair for the 2007-09 conferences that have served as venues for the dissemination of scholarship that impact training programs.

He has extensive evaluation and review experience. He is an external evaluator on several funded training programs, and through service on review panels for the National Institutes of Health, National Science Foundation, US Department of Agriculture and other non-federal organizations has reviewed numerous grants and served on several site visit teams in the assessment of many programs.

Shawn R. Drew

Dr. Shawn R. Drew joined the staff of the National Institute of General Medical Sciences at the National Institutes of Health (NIH) in September 2003 as a program director in the Minority Access to Research Careers Branch where she manages research grants, training grants, and fellowships aimed at increasing the number of underrepresented minority scientists. Dr. Drew also serves as the institute's program director for the Biostatistics Research Training Grant program and is the Chair of the Committee to Maximize Representation for research training grant programs. Prior to her appointment, she served as director of the NIH Academy, an intramural postbaccalaureate research training program, and was an adjunct professor of biology at the University of Maryland University College, College Park, MD and Prince George's Community College, Largo, MD. Dr. Drew earned a bachelor's degree in natural science with a concentration in chemistry in 1991 from Spelman College in Atlanta, GA and went on to earn a PhD in biology in 1998 from Howard University in Washington, DC, where she conducted her PhD dissertation research at the National Institute of Diabetes, Digestion, and Kidney Diseases (NIDDK), Laboratory of Chemical Biology. Her postdoctoral research was conducted in the NIDDK Molecular and Clinical Hematology Branch.

Jeff Ebersole

Dr. Jeff Ebersole is the Alvin L. Morris Professor of Oral Health Research, the Director of the Center for Oral Health Research, and Associate Dean for Research at the University of Kentucky College of Dentistry. He obtained his BS in Biology from Temple University and his PhD in Microbiology from the University of Pittsburgh. He held academic ap-

pointments through Senior Member of the Staff at The Forsyth Institute, Boston, Massachusetts, and as Associate Clinical Professor of Oral Biology and Pathophysiology at the Harvard School of Dental Medicine. He was then a Professor of Periodontics and Microbiology at The University of Texas Health Science Center at San Antonio, Texas. In 2000, he relocated to the University of Kentucky College of Dentistry. Dr. Ebersole is the PI for the Center for Biomedical Research Excellence in the College, which has just entered its second 5-year funding period. His research program focuses on the determination of inflammatory, innate and adaptive immune responses associated with colonization/infection of the oral cavity by selected periodontopathic bacteria. His research models include cell culture, small animals and nonhuman primates, and translational studies of oral diseases in various human populations. Dr. Ebersole is the author/co-author of over 200 peer-reviewed publications, books and book chapters, and the recipient of numerous fellowships and awards, including the Basic Research in Periodontal Disease Award from the International Association for Dental Research.

Sandra H. Glover

Dr. Sandra Glover is an Associate Professor in the Department of Health Services Policy and Management, and Associate Dean for Health Disparities and Social Justice in the University of South Carolina Arnold School of Public Health; Director of the USC Institute for Partnerships to Eliminate Health Disparities (IPEHD); Associate Director of the USC Rural Health Research Center; and Principal Investigator of the EXPORT Center for Partnerships to Eliminate Health Disparities in HIV/AIDS and Cervical Cancer. The EXPORT initiative brings together USC and Claflin University in research, education and outreach programs targeting health disparities at the two sites and around the state. Through the IPEHD and all the programs that she manages and oversees, as well as by virtue of her own research interest in health disparities and in promoting minority participation in the health sciences, Dr. Glover has developed a network for research and education that involves the University of South Carolina and all six HBCUs in the state.

Dr. Glover received her BA in Accounting at SC State College in 1979, her MBA and subsequently her PhD in Management and Organizational Behavior at the University of South Carolina, in 1984 and 1991, respectively. She was appointed to a 2-year National Research Service Award institutional grant for training and research with the South Carolina Department of Mental Health in collaboration with the University of South Carolina School of Medicine and School of Public Health, and then became a faculty member at the USC Arnold School of Public Health, where she was tenured at the rank of Associate Professor in August 2005. She served as Vice President for Research and Economic Development, and was Tenured Professor in the College of Business and Applied Professional Science at SC State University, while retaining a joint appointment with USC. She returned to her current position in the USC Arnold School of Public Health in June 2006.

Anthony Hayward

Dr. Anthony Hayward has directed the Division for Clinical Research Resources at the National Center for Research Resources since 2001. The Division for Clinical Research Resources at NCRR supports programs such as the Clinical and Translational Science Awards and the General Clinical Research Centers, as well as career development programs and specific initiatives supported through ARRA funding.

Dr. Hayward obtained a first degree in Physiology at University College, London, in 1964,

followed by a medical degree in 1967. He trained as a pediatrician at the Hospital for Sick Children at Great Ormond St in London and obtained a Ph.D. in Immunology in 1972. Between 1978 and 2001, he served on the faculty of the University of Colorado Health Sciences Center as Professor of Pediatrics, Microbiology and Immunology with appointments at National Jewish Center and The Children's Hospital. His research focused on immunity development, primary immunodeficiency syndromes and also on immunization strategies to reduce the burden of post-herpetic neuralgia. Dr. Hayward is author or co-author on over 160 original articles and has contributed to over 40 books.

Konstantin G. Kousoulas

Dr. Konstantin Kousoulas is the Director of the Division of Biotechnology and Molecular Medicine, and a Professor in the Department of Pathobiological Sciences at the Louisiana State University School of Veterinary Medicine. He obtained his BS degree in Physics at Fairleigh Dickinson University, Teaneck, NJ; his MS in Biophysics and his PhD in Molecular and Cellular Biology at Pennsylvania State University, University Park, PA. He held Postdoctoral Fellow positions at the California Department of Health in Berkeley, CA (as an ACS Fellow) and subsequently at UCSF, and at the University of Chicago, before moving to faculty positions at the University of California San Francisco, and at LSU, where he is currently Professor of Virology & Biotechnology. Dr. Kousoulas is currently the recipient of the Mary Louise Martin Professorship in Veterinary Medicine, and is an adjunct faculty member of the Department of Biological Sciences, College of Basic Sciences, and the Pennington Biomedical Research Center. In 1991, Dr. Kousoulas founded the Gene Probes and Expression Systems Laboratory "Gene Lab" in the Department of Pathobiological Sciences, which serves Louisiana researchers as a support and resource facility for research in molecular and cellular biology. GeneLab is now part of the Division of Biotechnology & Molecular Medicine (BIOMMED) in the LSU School of Veterinary Medicine. BIOMMED is currently administering the LSU-Tulane Center for Experimental Infectious Disease Research funded by the NIH COBRE mechanism (<http://www.labiomed.info>) and the Molecular/Cell Biology Core of the IDEa Network for Biomedical Research Excellence (INBRE). A recognized expert in the molecular biology and pathogenesis of herpes simplex viruses (HSV) and human herpesvirus 8 (HHV-8) or Kaposi's Sarcoma Associated Herpesvirus (KSHV), Dr. Kousoulas is author or co-author of over 72 publications and five patents, and has maintained over the years a vigorous, extramurally-funded research program that served as training grounds to numerous students, postdoctoral fellows, and young faculty members.

T. Scott Little

T. Scott Little, PhD is a Research Professor in the Department of Chemistry and Biochemistry at the University of South Carolina and Director of the State Office for South Carolina's Experimental Program to Stimulate Competitive Research (EPSCoR) and Institutional Development Awards (IDEA). He is responsible for an annual budget of \$10 million to develop the academic research infrastructure in South Carolina's institutions of higher education. Dr. Little has more than 100 peer reviewed publications, two book chapters, and one book on infrared, Raman, and microwave spectroscopic investigations of ring compounds. Dr. Little also has international research collaborations with the Central University of Antwerp in Antwerp, Belgium and the Indian Institute of Technology at Kanpur, India. He is the Managing Director of the South Carolina Research Center of Excellence for Regenerative Medicine and serves on the external advisory boards for four of South Carolina's eight Historically Black Colleges and Universities and for the

Department of Chemistry at Clemson University. Dr. Little has served as the Principal Investigator and Co-Principal Investigator on numerous Federal and State programs addressing K-16 STEM education for African-Americans living and being educated in rural settings. He has received numerous awards and honors for his work in chemistry and for providing greater access to research resources for underrepresented minorities. Dr. Little is a member of the American Chemical Society, the Society for Biomaterials and is certified in Advanced Evaluation Practice and Quantitative Methods by the Center for Evaluation Effectiveness at The George Washington University.

Curtis Lowery

The newly appointed Chairperson for the University of Arkansas for Medical Sciences' Department of Obstetrics and Gynecology, Dr. Curtis Lowery is viewed as a champion of antenatal and neonatal telemedicine benefiting the patient and physician alike. Dr. Lowery has served as the Director of Obstetrics at the University of Arkansas for Medical Sciences since 1992. In that time, he facilitated the process in which Arkansas insurance handles telemedicine, increased Medicaid reimbursements and promoted understanding for telemedicine, and brought telehealth access to over 60 hospitals and community clinics in rural Arkansas providing medical consultations combined with provider and patient education. Dr. Lowery has established a Medicaid-funded, cost-effective programmatic solution to assist Arkansas' high-risk pregnancies, ANGELS. Dr. Lowery founded this effort that reaches throughout Arkansas to those in need of subspecialty Maternal-Fetal Medicine support. In his latest effort, Dr. Lowery founded the UAMS Center for Distance Health, a technology-based partnership of the College of Medicine and Regional Programs. This Center directly offers telemedicine, continuing medical and health education, public health education, and evaluation research through interactive video throughout Arkansas. The Center for Distance Health represents the culmination of Arkansas' telemedicine and distance health technology expertise, with directors and stakeholders who have been instrumental in developing telehealth initiatives in Arkansas. He was recently recognized by the UAMS College of Medicine through the 2007 Educational Innovation Award, and ANGELS was announced by Harvard University Ash Institute as one of the nation's most innovative governmental collaborations. Dr. Lowery also received the 2007 Hugo Gernsback Award for Clinical Innovation in Telemedicine by the AT&T Center for Telehealth Research & Policy.

Sidney McNairy

Sidney A. McNairy Jr., PhD, is currently the Associate Director for Research Infrastructure in the National Center for Research Resources (NCRR), NIH and the Director of the Division of Research Infrastructure (DRI). Dr. McNairy came to NIH from Southern University in Baton Rouge, La., where he was the Director of the Health Research Center and a Professor of Chemistry. As the Director of DRI, he is responsible for providing oversight management for the Research Centers in Minority Institutions (RCMI) Program, the Institutional Development Award Program, an Animal Facilities Improvement Program, and the Research Facilities Improvement Program. From FY 02-08, Dr. McNairy managed over \$ 1.5 billion of federal funding that is now being used to conduct biomedical and behavioral research throughout the country. From 1994 to 2005, leveraging of Federal dollars via the facilities program resulted in projects totaling over \$2.1 billion, of which his division provided \$660 million, one third of the total investment made by the Federal government. From 2000 to 2005, the dollars appropriated for the RCMI and IDeA Programs was \$291 and \$946 million respectively. With these resources, these

communities were able to compete for \$15.45 and \$14 billion from the NIH during this same period.

Dr. McNairy earned his B.S. in chemistry/mathematics from LeMoyne-Owen College and both the MS and PhD degrees from Purdue University in biochemistry with minors in physiology and organic chemistry. During his graduate career he isolated, chemically characterized, and determined the molecular basis of the biological actions of triterpenoid glycosides. He has done further study at Columbia University. While a Professor at Southern he was a visiting scientist at Charles Pfizer, Eli Lilly, General Electric, Standard Oil of California, and the Centers for Disease Control and Prevention. One of the highlights of his research career was the time that he spent at Eli Lilly. He worked with the pioneering research team that isolated proinsulin, sequenced the insulin connecting polypeptide, and developed a radio-immunological assay for proinsulin.

Dr. McNairy has received numerous awards and honors, including 8 honorary doctorate degrees, designated an "Old Master" by his alma mater Purdue University. He is a member of the Golden Parade of alumni at LeMoyne-Owen College and was elected to the Board of Trustees of LeMoyne-Owen College.

He has received two University "Presidential Awards," the NIH's Director's Award, is a member of federal government's Senior Executive Service, and was selected by Harvard University's John F. Kennedy School of Government to participate in the Program for Senior Managers in Government. In 2002, he was the first recipient of the Frederick C. Greenwood Award, given in recognition of his meritorious service to the RCMI grantee community (RCMI grantees presently include 18 institutions that award doctorate degrees in the health professions and the biomedical sciences, located in 10 states, Puerto Rico, and the District of Columbia). He has been named an honorary member of the Tuskegee Veterinary Medical alumni Association. He has been designated an "Arkansas Traveler" by the governor of Arkansas. At the second biannual national IDEa Symposium Biomedical Symposium, a lecture series was named in his honor.

Vladimir A. Mironov

Vladimir A. Mironov, M.D., Ph.D. is an Associate Professor and Director of the Advanced Tissue Biofabrication Center in the Department of Regenerative Medicine and Cell Biology at the Medical University of South Carolina (MUSC) in Charleston, SC. He earned his MD in Medical Sciences at Ivanovo State Medical Institute in 1977 and his PhD in Histology/Embryology at Moscow Pirogov State Medical Institute in 1980. Dr. Mironov was trained in angiogenesis research at the Max Planck Institute in Germany.

Since 1993, he has worked at the Medical University of South Carolina, first as a post-doctoral fellow. He received an appointment as an Assistant Professor in the Department of Regenerative Medicine and Cell Biology in 2000, and has served as an Associate Professor in the same department since 2005. His research interests are cardiovascular development, vascular biology, tissue engineering. Dr. Mironov co-edited a book titled, "Vascular Morphogenesis: in Vivo, in Vitro, in Mente," and has authored or co-authored more than 100 peer-reviewed publications, including a recent, highly-cited paper entitled, "Organ printing: computer-aided jet-based 3D tissue engineering." Dr. Mironov was a finalist for the World Technology Award and received a prestigious Tan Chin Tuan Fellowship at The Nanyang Technological University, Singapore. Dr. Mironov holds 3 Russian and 2 American patents and he is a member of editorial board for three journals: "Biofabrication," "Journal of Angiogenesis Research," and "Expert Opinion on Biological Therapy." Further, Dr Mironov serves as a consultant for several biotech companies.

Elizabeth Ofili

Dr. Elizabeth Ofili is the Associate Dean, Clinical Research, Director of the Clinical Research Center, a Professor of Medicine and Chief of Cardiology at Morehouse School of Medicine. Dr. Ofili completed medical school at Ahmadu Bello University in Nigeria and received her Masters in Public Health at John Hopkins University in Baltimore, Maryland. After completing her Internal Medicine Residency Program at Oral Roberts University in Tulsa, Oklahoma she pursued her Cardiology Fellowship at Washington University and St. Louis University Health Science Center, St Louis MO.

Dr. Ofili has an active interest in the mechanism of myocardial dysfunction with particular emphasis on the role of ultrasound imaging modalities. She received the Young Investigator Research Award early in her career for her work on the physiological stress agents in a canine model of coronary artery disease. In collaboration with Dr. Morton Kern, Dr. Ofili developed and validated the method of analysis of the intracoronary doppler spectral wave form used to study coronary flow reserve. She is recognized for her expertise in the field of echocardiography in clinical and population based trials.

Dr. Ofili is a past President of the Association of Black Cardiologists, Inc., an active member of the International Society of Hypertension in Blacks and serves on several national and international committees. Dr. Ofili has published over one hundred scientific papers, book chapters and abstracts, and has over 250 scientific presentations. A recipient of the Preventive Cardiology Academy Award, Dr. Ofili established large clinical patient databases at Grady Memorial Hospital in congestive heart failure, chest pain and hyperlipidemia. These databases allow evaluation of treatment effectiveness, as well as a patient enrollment resource for ongoing clinical trials in coronary artery disease, hyperlipidemia, hypertension and congestive heart failure. Dr Ofili is the principal investigator of the NHLBI funded Heart Failure Network Regional Coordinating Center at Morehouse School of Medicine, and Co-PI of the Atlanta Clinical and Translational Science Institute, a three institution CTSA partnership between Emory University, Morehouse School of Medicine and Georgia Institute of Technology.

Lucia Pirisi-Creek

Lucia Pirisi-Creek, born in Italy in 1954, married and the mother of two, is Professor of Pathology, Microbiology and Immunology at the University of South Carolina School of Medicine. She received her MD degree in Italy in 1983, and moved to the USA in 1985 as an NIH Fogarty Visiting Scientist to conduct basic cancer research at the National Cancer Institute. In August, 1988, she became an Assistant Professor of Pathology at the USC School of Medicine, where she rose through the ranks to her current position. Dr. Pirisi-Creek is the author or co-author of 58 scientific publications, and has mentored numerous PhD, master, and undergraduate students. Dr. Pirisi-Creek co-directs with her husband and collaborator, Dr. Kim E. Creek, an active, extramurally-funded research program focusing on cervical cancer and its major causative agent, human papillomavirus (HPV). She also has a research interest in the breast cancer field. In 2004, she became Senior Faculty Associate for Biomedical Research with the Vice President for Research at USC, and in that capacity, served as Interim Deputy Director of the Research Division of the South Carolina Cancer Center for two years. She currently directs the SC INBRE program, and is Secretary/Treasurer of the National Association of IDeA Principal Investigators. Dr. Pirisi-Creek is a founding member, and the Secretary/Treasurer of the International Papillomavirus Society, and President of the South Carolina Academy of Science. Among Dr. Pirisi-Creek's interests outside of science are music (piano), poetry and dance. In 2003, she spearheaded the formation of the South Carolina Multicultural

Arts Center, Inc. (www.scmcac.org), of which she is President. Through the SC McAC, she has organized and directs "Artists Against Breast Cancer," a research, education and outreach program that uses the arts to promote breast cancer awareness.

Lili M. Portilla

Ms. Lili Portilla has worked in the area of technology transfer at the National Institutes of Health since 1989. She has extensive experience in negotiating and developing commercialization strategies for complex, and multi-party collaborations. Ms. Portilla has broad knowledge of federal and NIH technology transfer policy and law pertaining to biotechnology and commercialization issues. Since January 2008, Ms. Portilla has served as Senior Advisor to the Director of the National Center for Research Resources (NCRR) and provides advice to NCRR staff on all facets of technology transfer, intellectual property and public private partnerships issues. She currently serves as technology transfer advisor for the NCRR funded Mutant Mouse Regional Resource Consortium and the National Swine Research Resource Center programs. She also works closely with the NCRR funded Rare Diseases Clinical Research Network to bring in proprietary drugs and compounds into the network. Ms. Portilla is the current NCRR Co-Chair of the CTSA Public Private Partnership Committee.

Prior to her position at the NCRR, Ms. Portilla served as the Director of the National, Heart, Lung and Blood Institute (NHLBI), Office of Technology Transfer and Development (OTTAD). Ms. Portilla received a Masters in Public Administration in 1992 from American University, Washington, DC and a Bachelor in Business Administration, double major in both Finance and Spanish Literature in 1986 from Stephen F. Austin State University.

Karin Remington

Dr. Karin Remington is Director of the NIGMS Center for Bioinformatics and Computational Biology, where she oversees research and training grants to support projects that integrate biology with computer sciences, engineering, mathematics, and physics. Before joining NIH, Dr. Remington served as the project manager for the National Ecological Observatory Network, or NEON, Inc., an effort supported by the National Science Foundation to construct ecological data collection facilities across the contiguous United States, Hawaii, Alaska, and Puerto Rico. As vice president of bioinformatics research at The Venter Institute from 2002 to 2006, she led an NIH-supported large-scale genome sequencing production center and spearheaded a traveling laboratory-based educational program for public school students in Washington, D.C. At Celera Genomics from 1999 to 2002, Dr. Remington developed mathematical methods and computation leading to the completed sequences of the fruit fly, human, and mouse genomes.

Judith Salley-Guydon

Dr. Judith Salley-Guydon is the chairperson of the Department of Biological and Physical Sciences at SC State University. She serves as the Executive Director of one of South Carolina's oldest and most successful alliances, the Louis Stokes South Carolina Alliance for Minority Participation Program (LS-SCAMP), where she oversees the management and coordination of programs at 12 alliance colleges and universities. Funded by the National Science Foundation, SCAMP's mission is to increase the number and academic performance of underrepresented minority students who earn BS degrees in the science, technology, engineering and, mathematics disciplines. Located in over 41 states, the LS-AMP program was recognized by *Diverse Magazine* in September 2008 as one of

the top 10 Diversity Champions in the nation.

Michael H. Sayre

Dr. Michael Sayre is a senior program official at the NIH National Center for Research Resources, where he manages a diverse portfolio of grant programs to strengthen the nation's biomedical research capacity and broaden participation in clinical and translational research. Prior to joining NCRR, he served as a scientific review officer at the NIH Center for Scientific Review, managing grant reviews in areas of molecular genetics, microbiology and infectious diseases, and cell and developmental biology, as well as research training programs for under-represented minority students. He earned his B.S. degree in botany and plant pathology from Oregon State University and his Ph.D. degree in biology (molecular genetics) from the University of California, San Diego. His postdoctoral research training at Stanford University focused on fundamental mechanisms of gene expression in eukaryotic organisms. As a faculty member at the Johns Hopkins University School of Public Health, he studied basic mechanisms of messenger RNA synthesis and developed a graduate teaching curriculum in molecular genetics and cell biology. His research has been supported by the National Science Foundation, the American Cancer Society, the National Institute of General Medical Sciences, and the National Cancer Institute. He has authored or co-authored more than 30 peer-reviewed publications, book chapters, abstracts, and reports, has presented his work at scientific conferences and invited seminars nationwide. He has served as a peer reviewer for front-line research journals and grant review panels for the NIH, the U.S. Army Breast Cancer Research Program, and private-sector research foundations.

W. Fred Taylor

W. Fred Taylor serves as the Director of the Institutional Development Award (IDeA) Program in the Division of Research Infrastructure of the National Center for Research Resources at the National Institutes of Health. He provides a broad range of technical, scientific, and health-related support to extramural program administration. He is responsible for planning and implementing a national program to enhance and improve the biomedical and behavioral research capacity and infrastructure in IDeA eligible institutions. Dr. Taylor's specific duties involve oversight and management of the COBRE (Centers of Biomedical Research Excellence) initiative, the INBRE (IDeA Networks of Biomedical Research Excellence) initiative, the co-funding activities sponsored through the IDeA program and the IDeANET initiative. He also participates in other program activities such as the Research Centers in Minority Institutions and Research Facilities Improvement programs.

Dr. Taylor's early career research interest was cardiac electrophysiology and the ventricular conduction pattern in avian hearts. He then studied the neural control of the circulation with emphasis on the impact of environmental stress and exercise on the peripheral circulation (i.e. skin and muscle circulations) with emphasis on the active thermoregulatory vasodilator system in the human cutaneous circulation. While at the Naval Medical Research Institute, Dr. Taylor served as the Program Director of the Hyperbaric Environmental Adaptation Program and as Deputy Director of the Thermal Stress Program in The Department of Diving and Environmental Physiology. His research interests at the Department of Defense included pharmacological, dietary, and training interventions for augmenting work capacity and cold tolerance (particularly cold acclimation). He studied the stress of hyperbaria and cold-water environments as they present extreme cardiovascular and thermal challenges that may seriously degrade performance and increase the risk of

morbidity and mortality.

Dr. Taylor holds a bachelor of science degree in Biology from Saint Mary's College of California, a master of arts degree in biology from California State University Sacramento and a master of science and doctorate in physiology from the University of Texas Health Science Center in San Antonio.

John Wheeler

Dr. John Wheeler serves as Chair of the Office of Integrative Research in the Sciences and Professor of Chemistry at Furman University. After receiving a B.S in Chemistry from a summa cum laude graduate of Georgetown College in 1986, Dr. Wheeler completed his Ph.D. in Analytical Chemistry under Professor William R. Heineman at the University of Cincinnati in 1990 and served as a postdoctoral scholar under Professor G. John Dorsey before beginning in his teaching/research career at Furman in 1991. Dr. Wheeler was selected as a Henry Dreyfus Teacher-Scholar by the Camille and Henry Dreyfus Foundation in 2000, and named the Henry Keith and Ellen Hard Townes Associated Professor of Chemistry at Furman in that same year. He was promoted to the rank of Professor in 2003, and named as Furman's first Director of Integrative Research in the Sciences in 2008.

Professor Wheeler's research interests lie in a broad range of applications in bioanalytical chemistry, and he and his students (including 12 M.S. students and over 100 undergraduates) have been supported NSF, NIH and several private foundations during his tenure at Furman. Dr. Wheeler served as co-Director of Furman's NIH-BRIN initiative from 2002-2004, and as Director of Furman's NIH-INBRE initiative from 2005-2010. He also serves as the PI and director of Furman's HHMI-USE award from the Howard Hughes Foundation (2008-2012), as the PI and co-Director of Furman's Merck/AAAS USRP program (2005-2008, 2009-2012), and as Director of Furman's NSF-RII initiative, a part of the South Carolina NSF RII EPSCoR award (2009-2014). In each of these programs, service to underrepresented and/or disadvantaged groups including enhancing diversity within the professoriate, providing undergraduate research opportunities and stimulating K12 STEM outreach is a significant programmatic goal, often involving students from South Carolina historically black institutions in addition to participants from the Furman campus.

Cynthia Wright

Cynthia Wright, PhD, is Associate Professor of Microbiology and Immunology at MUSC and Assistant Dean for Admissions, College of Graduate Studies. She received her undergraduate training as a Microbiology major at the University of Florida and her graduate training at SUNY-Albany, supported by a prestigious NSF predoctoral fellowship award. She undertook postdoctoral training at the NIH, supported by an individual NRSA award. During her career, Dr. Wright has been funded by grants from the NIH, NSF, DOD, US Army, and the Elsa U. Pardee Foundation. Her research work has focused on the regulation of gene expression in viruses and cancer. Currently, Dr. Wright directs three NIH-funded training programs, all of which are related to diversity initiatives. These include an NHLBI-funded R25 program "Short-Term Training for Minority Students" that supports undergraduate students to perform research on the MUSC campus in the summer. Dr. Wright is also the Program Director of the NIGMS-funded MUSC IRACDA program, which provides a mentored research and teaching experience for postdoctoral fellows. This program is done in conjunction with Claflin University, which is where the

teaching externship occurs. Finally, Dr. Wright is also the Program Director for the MUSC Initiative for Maximizing Student Diversity (IMSD) program, also funded by NIGMS. The goal of the IMSD program is to enhance the training environment for students during their years in graduate school through classes, workshops, and mentoring groups. In addition to directing training programs, Dr. Wright continues to be active in teaching. She serves as the course director for the Diversity in Science class, directs and teaches in the laboratory portion of the summer pre-matriculation course, and teaches in the didactic portion of the interdisciplinary curriculum for first-year PhD students. The graduate students have nominated Dr. Wright several times for Outstanding Teacher of the Year.

Thomas R. Ziegler

Thomas R. Ziegler, M.D. is a Professor of Medicine in the Department of Medicine, Division of Endocrinology, Metabolism and Lipids at the Emory University School of Medicine. His research involves clinical and translational studies relevant to specialized nutrition support in critical care, catabolic illness and intestinal failure and emerging work in redox-related mechanisms and nutritional metabolomics. He serves as the Director of the Emory Center for Clinical and Molecular Nutrition. In his CTSA administrative functions, Dr. Ziegler is Co-Program Director for the Research Education, Training and Career Development Core of the Atlanta Clinical and Translational Science Institute (ACTSI), Program Director of the Emory CTSA/ACTSI TL1 component grant and Co-Program Director of the ACTSI KL2 grant. He also serves as the Director of the Emory University Hospital Clinical Interactions Site within the ACTSI Clinical Interactions Network.