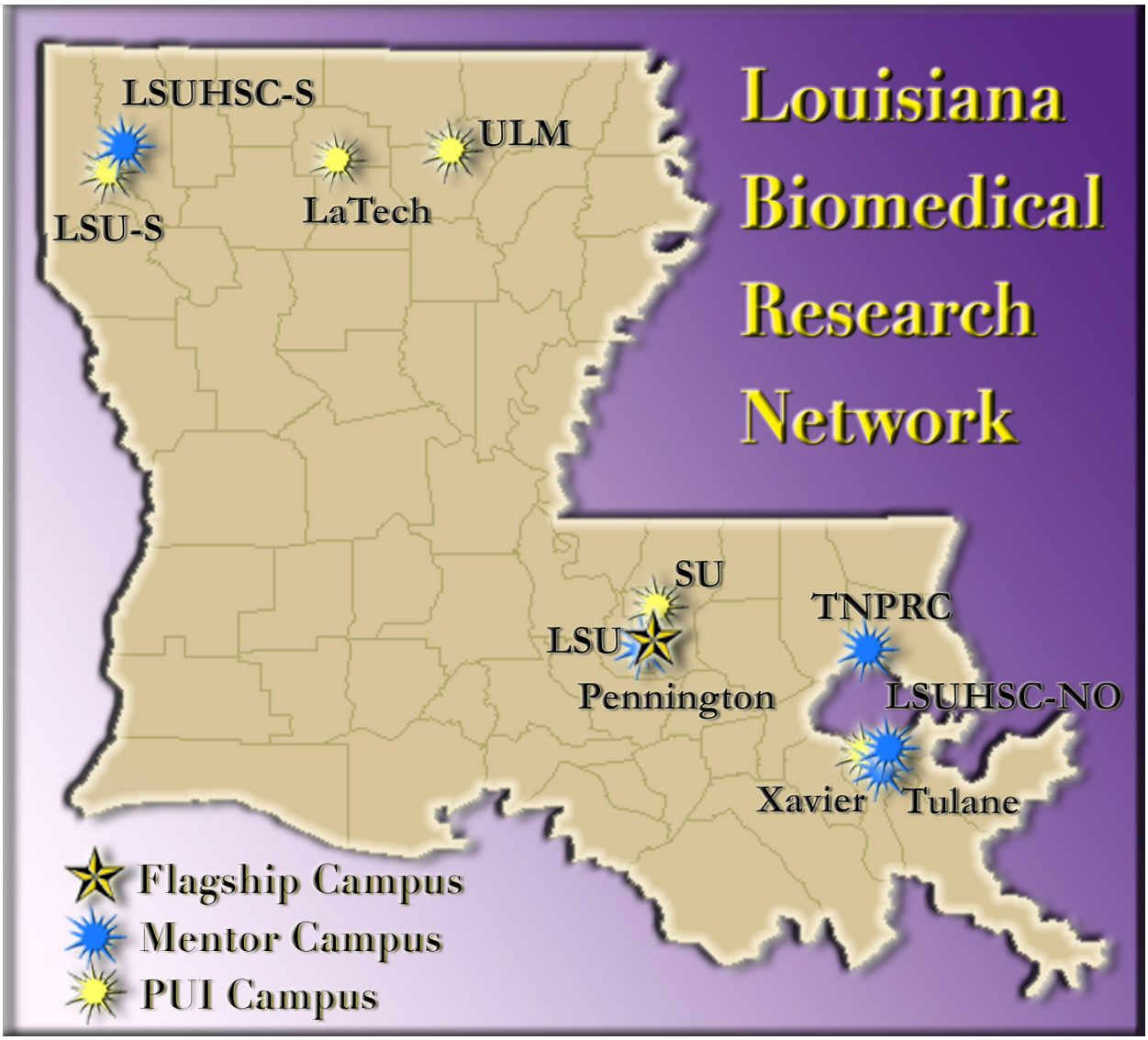


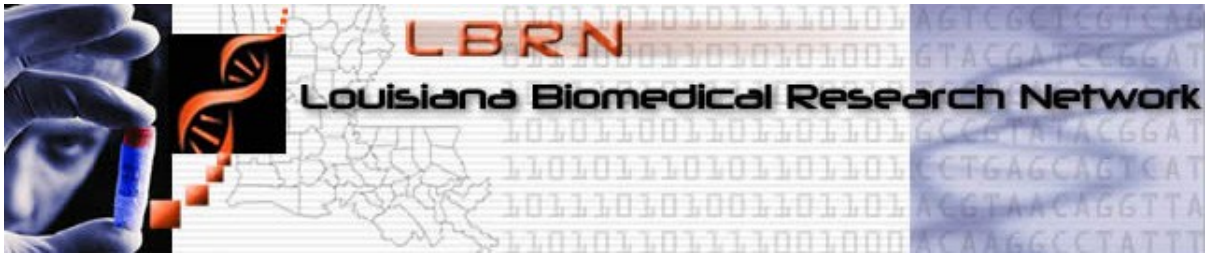
Louisiana Biomedical Research Network

11th Annual Meeting

Louisiana Tech University

Ruston, LA

January 18-19, 2013



Administrative Structure

Administrative Core

Thomas Klei (PI)

Bill Wischusen (PC)

Bioinformatics, Biostatistics, & Computational Biology Core

Brygg Ullmer

Hilary Thompson

External Advisory Committee

Vincent McKoy (Chair)

Richard Hart

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Stan Napper

Mario Philipp

Robert Rhoads

Paul Sisson

Michael Stubblefield

Hilary Thompson

E. William Wischusen

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Agenda

FRIDAY, JANUARY 18, 2013

	Meeting Room	
4:30 - 5:30 pm	University Hall Foyer	**Meeting Registration / Poster Set-up / Hotel Check-in**
6:00 - 7:00 pm	University Hall Foyer	Welcome Dinner
7:00 - 9:00 pm	University Hall, 111	Poster Session (with coffee and desert)

SATURDAY, JANUARY 19, 2013

		Meeting Room	Research Presentation	Meeting with EAC Univ. Hall 123
	7:00 - 8:00 am	University Hall, 111	***Breakfast***	
	8:00 - 8:15 am	University Hall, 134	***Introduction***	
	8:15 - 8:35 am	"	**Teresa Murray[3]**	
	8:13 - 8:35 am	"	**Thomas Huckaba[3]**	
	8:55 - 9:15 am	"	**Amal Kaddoumi[4]**	
Concurrent Session 1	9:15 - 9:30 am	"	Quincy Quick[2]	**Murray & mentor**
	9:30 - 9:45 am	"	Rebecca Giorno[2]	**Huckaba & mentor**
	9:45 - 10:00 am	"	Adarsh Radadia[2]	**Kaddoumi & mentor**
	10:15 - 10:35 am		***Break***	
	10:35 - 10:55 am	University Hall, 134	**Tara Williams-Hart[4]**	
	10:55- 11:15 am	"	**Eduardo Martinez Ceballos[4]**	
	11:15 - 11:30 am	"	**Harris McFerrin[4]**	
Concurrent Session 2	11:30 - 11:45 pm	"	Joseph Olabadewo[2]	**Williams-Hart & mentors**
	11:45 - 12:00 pm	"	Kui Chen[2]	**Martinez Ceballos & mentor**
	12:00 - 12:15pm		Hisham Qosa[1]	**McFerrin & mentor**
	12:15 - 1: 30 pm	University Hall, 111	***Lunch***	

Agenda cont.

SATURDAY, JANUARY 19, 2013 continued…

		Meeting Room	Research Presentation	Meeting with EAC Univ. Hall 123
	1:30 - 1:50 pm	University Hall, 134	**Elahe Mahdavian**[4]	
	1:50 - 2:10 pm	"	**Prerna Dua**[4]	
Concurrent Session 3	2:10 - 2:30 pm	"	Abhita Malaviya[1]	Mahdavian & mentor
	2:30 - 2:45 pm	"	Mihir Karnik[1]	Dua & mentor
	2:45 - 3:00 pm	"	Kankana Shukla[1]	
	3:00 - 3:15 pm	University Hall, 111	***Break***	
	3:30 - 3:50 pm	University Hall, 134	**Seetharama Satyanarayanajois**[4]	
	3:50 - 4:10 pm	"	**Shuju Bai**[4]	
Concurrent Session 4	4:10 - 4:25 pm		Xiaoping Yi[1]	Satyanarayanajois & mentors
	4:25 - 4:40 pm		Ameya Gokhale[1]	Bai & mentors

[1]Summer Graduate Student, [2]Summer Faculty, [3]Pilot Project PI, [4]Project PI

6:00 - 7:00 pm	Squire Creek, The Gathering Room	Reception
7:00 - 9:00 pm	Squire Creek, The Gathering Room	Dinner

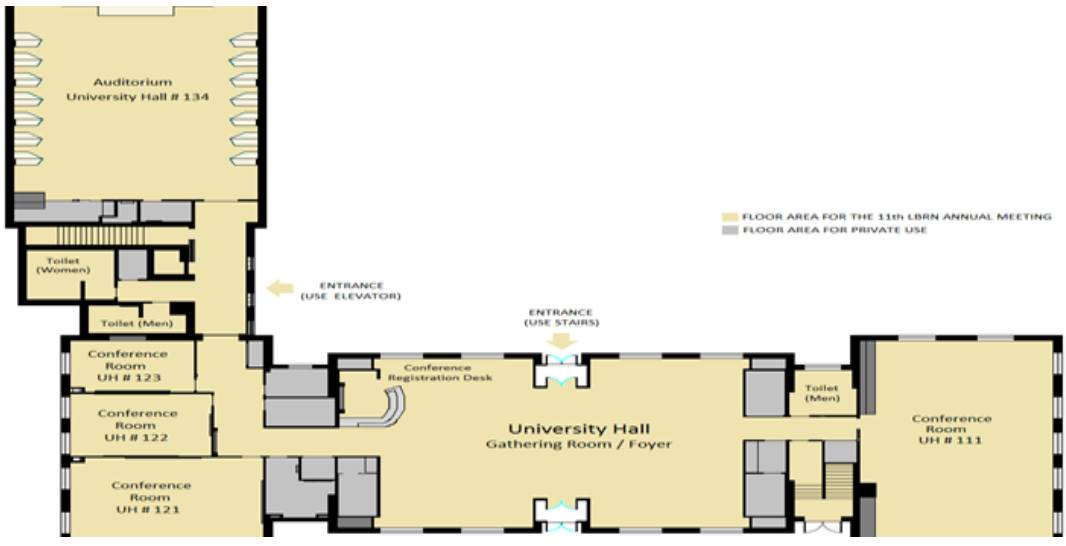
Squire Creek Country Club

289 Squire Creek Parkway, Choudrant, LA

Marriott Fairfield Hotel

1707 Roberta Ave, Ruston, LA 71270

Meeting Floor Plan



University Hall

Notes

Poster Session Abstracts

Poster Session Abstracts

1 Identification of FC101 protein interactions by affinity column chromatography

Steven Adelmund, Dr. Tara Williams-Hart and Dr. Elahe Mahdavian
Louisiana State University - Shreveport

Fusarochromanone (FC101) is a potential new drug that shows promise in treating a variety of cancers. The chemical appears to affect cancer cells differently than other common treatments. FC101 has demonstrated no innate toxicity at lower doses. There is an apparent preferential uptake of FC101 into the cancer cell lines when compared to normal cell lines, providing a broad therapeutic window. In cancer cells the drug is concentrated in the endoplasmic reticulum, the perinuclear cytoplasm, lysosomes, and the Golgi apparatus. Despite its discovery in 1985, the mechanism of FC101's function is still undetermined. The purpose of this research is to develop an understanding of FC101's mode of action using affinity column chromatography. FC101 will be coupled with biotin through an esterfication reaction. The resulting FC101-biotin ligand will be bound to an avidin column. Cancer cell fractions will be run through the column, and proteins that interact with FC101 will be retained by the column. Bound proteins can be removed by a variety of elutes. Eluants will be collected and purified using gel electrophoresis. Proteins will be identified using mass spectroscopy. The results of this study will provide insights into the mechanism of FC101 by identifying the protein interactions of the drug in cancer cells. From this more effective treatment methods can be developed and functional group alterations of FC101 can be proposed that will potentially increase its efficacy.

2 Role of IunH in Bacillus anthracis spore germination

Rebecca Giorno-McConnell, C. Zhang and E. Dill
Louisiana Tech University, Grambling State University

Bacillus anthracis spores are the infective particle of the disease anthrax. Our goal is to understand the role of the exosporium in germination and dormancy. Although dormant, spores can resume metabolic activity in the presence of nutrients. This process, called germination, results in the shedding of the outer spore protective structures (the exosporium and the coat) and the return to vegetative growth. In Bacillus thuringiensis, germination in response to inosine can be controlled by the abundance of a spore-specific inosine hydrolase (IunH). Therefore, we investigated the role of IunH in B. anthracis to determine if it would influence the ability of spores to germinate in response to inosine. Germination in B. anthracis can be triggered using a combination of two germinants, a nucleoside and an amino acid. Using a combination of the nucleoside inosine and either alanine, serine, or tryptophan, we compared the spore germination kinetics of wild-type B. anthracis versus an iunH mutant. We also measured the inosine hydrolase activity of iunH mutant spores compared to wild type. We found that the absence of the exosproium protein IunH can increase the germination rate in response to inosine with any above amino acid as well as decrease the threshold concentration of inosine required to trigger germination. We also found measurable IunH activity in wild type spores and no detectable IunH activity in iunH mutant spores. These data suggest IunH influences germination kinetics by degrading inosine. This in turn allows persistence and/or survival of B. anthracis in the environment or during infection by preventing the spores from germinating when there is limited nutrition.

3 Understanding the Role of SpoVID and CotO in the Proper Assembly of the Coat and Exosporium of Bacillus anthracis

Elizabeth Griggs, Trey Hanna and Rebecca Giorno
Louisiana Tech University

Spore formation in Bacillus anthracis occurs when the bacterium is met with a nutrient poor environment. When this happens, the bacterium undergoes a transformation from the gram positive, rod form, to the more protective spore form. The spore is composed of genetic material housed by the cortex, and surrounded by a protective shell called the coat. The coat

protects the cortex from small toxic molecules such as lysozyme. In addition, B. anthracis also has a loosely fitting shell called the exosporium. Some genes which control the formation of the coat in B. anthracis are also thought to control the formation of the exosporium. The goal of this research is to complement spoVID and cotO mutant strains of B. anthracis. The cotO mutant forms no exosporium and the spoVID mutant has very little coat formation, but maintains an exosporium. In order to show if disruption in spore morphology is caused by the mutated gene, a complementation of the spoVID and cotO mutant strains will be performed. Because spoVID is in a two gene operon with ysxE, either spoVID or spoVID ysxE will be used to complement the spoVID mutant strain. Similarly, cotO will be added to the cotO mutant strain to see if the addition of the gene will revert back to wild type phenotype. In addition, inosine hydrolase activity will be compared in wild type, spoVID, cotO, and IunH mutant spores, to see if morphological changes correlate with loss of inosine hydrolase activity.

4 Use of Optical Density and Colony Forming Units to Determine the Efficacy of Antifungal Drugs on Candida albicans

Patrick Hindmarsh, Hunter Collins and Edward Pierce
Louisiana Tech University

Candida albicans a unicellular budding yeast is a common constituent of the human flora and resides in the guy mouth and other mucosal surfaces. C albicans is responsible for a number of human diseases and has become an important medical issue. C. albicans is the fourth most commonly acquire hospital infection. For the healthy individuals, C. albicans has a benign association but for others in particular those who are hospitalized or immunocompromised C. albicans can be highly pathogenic. Our group has developed an assay system to identify antifungal drugs in combination to control growth in candida albicans. Several antifungal drugs are presently used to control c albicans growth in infected individuals. Control of C. albicans infections face two obstacles, toxicity to the infected individual and the development of resistance by c albicans to the present antfungal therapies. Our goal is to identify combinations of antifungal drugs that decrease c albicans growth using sub-optimal concentrations with the goal of reducing resistance and lowering host toxicity. We have been using growth curves to identify synergistic activity using several two-drug combinations. We also plated out our treated samples after a series of dilutions to determine the number of viable cells (colony forming units) remain after exposure to the antifungal drug combinations. By using these tests in combination we are able to more accurately show the efficacy of the drug treatments.

5 Development of an Autonomous Replicating Plasmid for Candida albicans

Patrick Hindmarsh, Xuan Li and Zach Allgood
Louisiana Tech University

Candida albicans is a dimorphic fungus that is part of the normal human flora. A suppression of the immune system causes overgrowth of C. albicans causing an infection, candidasis. Candidasis can be a severe or deadly infection in the immunocompromised. The increase in candidasis as a nosocomial infection and the ineffectiveness of current medications creates a need for new genetic tools to investigate C. albicans. Plasmids currently used to study C. albicans are unstable, form large tandem multimers, and integrate into the genome. The goal of this project is to develop an autonomous replicating plasmid to serve as a genetic tool for analysis of C. albicans transcription, regulation, pathogenesis, and infectivity. The newly developed plasmid will have a segregation system to prevent the problems seen in current plasmids. The segregations system for the plasmid will consist of Epstein Barr Nuclear Antigen 1 (EBNA1) and the Family of Repeats (FR) sequence; both components are involved in segregation and replication of Epstein Barr Virus (EBV). The FR sequence from plasmid pAPG26 has been cloned into the vector containing the C. albicans autonomous replicating sequences, plasmid pMK22. Once generated, pMK22FR will contain the FR region and the C. albicans autonomous replicating sequence. To generate this plasmid we will be using colony PCR, plasmid DNA purification and extraction, restriction digestions, and ligation.

6 Characterization of Hereditary Spastic Paraplegia-Causing Mutations in Kinesin Kif5A

Thomas Huckaba, David Nathan, Davon Carter, Edward Wojcik and Sunyoung Kim
Xavier University of Louisiana, LSUHSC - New Orleans

Hereditary Spastic Paraplegias (HSPs) are a group of neurodegenerative disorders that arise from the progressive degeneration of corticospinal tract axons, causing lower limb spasticity and weakness. An autosomal dominant form of HSP (AD-HSP) is caused by mutations in Kif5A, a neuronally enriched form of the kinesin-1 family of cellular transport motors. While nineteen separate AD-HSP-causing missense mutations in Kif5A have been mapped, eighteen are in the motor domain, suggesting a deficit in catalytic activity as the mechanistic cause of the disease. We have mutated the wild type Kif5A gene with each of the separate AD-HSP-causing mutations and have begun to test the mechanical properties of these recombinantly-expressed mutant motors in a series of in vitro biochemical and biophysical assays. Here we report the results of four separate mutations in the ATP binding and hydrolysis pocket (S202N, S203C, R204W, and V231L) and one mutation in the L11 loop predicted to be at the microtubule interface (E251K). Performing microtubule pelleting assays in the presence of saturating levels of ATP, we found that S202N and S203C had a significantly higher microtubule affinity than wild type Kif5A or the other mutants. Conversely, in the presence of the non-hydrolyzable ATP analog AMPPNP, R204W, V231L and E251K had a significantly lower microtubule affinity than wild type and the other mutants. In microtubule gliding assays, wild type Kif5A moved microtubules at a rate of 0.47 ± 0.02 μm per second. The S203C mutant moved microtubules at a 60-fold slower rate of 0.0074 ± 0.0028 μm per second, while the S202N, R204W, and E251K mutants bound microtubules to the glass surface in rigor and the V231L mutant was unable to recruit microtubules from solution. These contrasting results suggest that AD-HSP may be caused by a variety of different mechanical deficits in the kinesin catalytic core.

7 Interaction of The Plant Cell Cycle Inhibitor with Specific Cyclins and Cyclin Dependent Kinases

Elisa James, Narender Kumar, Alice Simmons and Dr. John C. Larkin
Grambling State University and Louisiana State University

During the development of multicellular organisms, cell differentiation is tightly coordinated with cell division. In animals, loss of control of cell division leads to cancer. In some cell types of both plants and animals, a modified cell cycle occurs during differentiation in which the DNA is replicated without concomitant cell division, resulting in an increase in nuclear DNA content. This process is called endoreplication; in plants, important examples of endoreplicated cell types include cereal endosperm, cotton fibers, and nitrogen-fixing symbiotic nodules in legumes. Endoreplication plays important roles in for cell differentiation, and is essential for nitrogen fixation in legume root nodules, but the detailed mechanism of this modified cell cycle remains poorly understood. Previous work using Arabidopsis leaf hairs (trichomes) as a model for cell differentiation demonstrated that the SIAMESE (SIM) gene encodes a cyclin-dependant kinase inhibitor that suppresses mitosis during establishment of endoreplication.

Plants have many different Cyclins and CDKs and we already know some interaction between SIM with different CYC/CDKs in Bimolecular Fluorescence Complementation (BiFC). The aim of this project is to test the hypothesis that CYCB2;4 interacts with SIM and CDKB1;1in BiFC.

8 Co-Expression and Differential Expression Analysis of miRNA regulation in Alzheimer's

Arunkumar Junuthula, Prerna Dua and Walter J. Lukiw
Louisiana Tech University and Louisiana State University

Alzheimer's disease is a progressive disease which causes the loss of brain function leading to loss of intellectual ability and eventually resulting in death. It is the most common form of dementia with no known cure however. A lot of factors can be attributed to Alzheimer's disease and research at the genomic level has led to the identification of various miRNAs which could possibly play a crucial role in the cause and progression of this disease. Recent molecular evidence indicate

that at least 4 miRNAs - including the NF-κB-regulated miRNA-9, miRNA-125b, miRNA-146a and miRNA-155 - are progressively up-regulated in both AD and AMD. We have evaluated the co-expression and differential expression patterns of these miRNAs with specific emphasis on the quartet group. Evaluation of co-expression patterns facilitates the discovery of the interactions and correlations between these miRNAs. This would allow us to identify and isolate miRNAs which are expressed simultaneously to provide more insights of their effects on the disease. Differential expression allows us to visualize any significant relative change in miRNAs before and after the advent of the disease in patients. This process could potentially identify and isolate the miRNAs which have a role to play in the disease. The dataset used consists of 5 high quality control whole brain samples, 5 high quality temporal brain samples and 5 high quality hippocampal brain samples with 1922 miRNAs. We have identified miRNAs which have significant co-expression and differential expression patterns based on Pearson correlation coefficient and Z-scores to detect and visualize the intensity dependent ratio of microarray data.

9 Design of cyclic and D-amino acid containing peptidomimetics for inhibition of protein-protein interactions of EGFRs and its implication in xenograft model of breast cancer

Shanthi Kanthala, Sashikanth Banappagari, Ameya Gokhale, Yong Yu Liu and Seetharama D. Satyanarayanajois
University of Louisiana at Monroe

HER-2 (Human Epidermal growth factor Receptor 2), a member of EGFR (Epidermal Growth Factor Receptor) family is a cell membrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. It is encoded by ERBB2 gene whose amplification leads to overexpression of HER-2 is correlated with higher aggressiveness in approximately 30% of all breast cancers. Previously, a peptidomimetic compound 5, designed based on the crystal structure of HER-2 and Herceptin has been reported to have antiproliferative activity in the nanomolar range. The objective of this study is to derive the structure activity relationship of cyclic and D-amino acid peptidomimetics designed thereafter. To evaluate the anti-proliferative activity of the designed compounds, CellTitre-Glo assay was performed on three breast cancer cell lines SKBR-3, BT474 which overexpress HER-2 and MCF-7 cells that do not express HER-2. The analogues of compound 5 with good activity were selected and a florescence assay was performed to assess the competitive binding to HER-2 overexpressing cells. Further, in vivo activity of the compounds was determined using xenograft model of human breast cancer in athymic nude mice.

10 Simulating Protein-Substrate Interactions in 8R-Lipoxygenase using Computational Approaches

Zhongwei Li, Ebrahim Khosravi and Shuju Bai
Southern University and A&M College

Lipoxygenases family (LOX) is present in a wide variety of organisms, including mammals, plants, and bacteria. It is believed to be the major cause of pathological symptoms in asthma by biosynthesis of leukotrienes. The physiological function is known as firstly producing HPETE (derived from arachidonic acid), which is transformed in further enzymatic step into leukotrienes. However, much less detail is known about the role of LOX in the inflammatory reaction.

In this research, the interaction of protein-substrate in 8R-lipoxygenase, a member of the lipoxygenase (LOX) enzyme superfamily was modeled. High performance computing was used to perform protein structure refinement, force field scheme development, and molecular dynamic simulation. The results of the research suggest a general model with sets of force field parameters, which is stable for MD simulation and could provide possible explanation of the interactions between 8R- lipoxygenase and its substrate, arachidonic acid. The model will also help drug design targeting human 5-LOX.

11 Analysis of differentially expressed and co expressed genes in the miRNA data for Alzheimer's disease

Krishna Kalyani Lingamdinne
Louisiana Tech University

Alzheimer's disease (AD) is one of the most common form of dementia. It is a very aggressive brain disorder that attacks the brain and results in loss of memory, and impaires cognitive behavior and subsequently leads to death. Gene expression profiling for Alzheimer's disease is necessary for better understanding of the variety and integration of multiple physiological changes in the brain. Identification of differentially expressed genes provides the list of most significant genes that plays a major role in the disease.

The current Alzheimer's microarray analysis is to identify the set of genes that are differentially expressed (DE) and co expressed in different biological states (diseased, normal) by using various microarray software tools available. Related research postulates the evidence of several statistical methods and algorithms that are applied in order to find differentially expressed and co-expressed genes from the miRNAs. Our current research focuses on three different brain regions of control, temporal lobe and hippocampus. In our present study, an experimental investigation of four different algorithms namely SAM (Significance Analysis of Microarray), MeV (MultiExperiment Viewer) and Cyber-T are performed to identify differentially expressed genes. Further we investigated to discover the clusters of co-expressed miRNAs. An exhaustive literature search is performed on the differentially expressed and the co-expressed miRNAs.

12 Inhibition of HSV-1-associated ocular neovascularization by flavopiridol and DRB

Harris McFerrin, Tatyana T. Santoke, Kenneth F. Swan, Elise I. LeMelle, Willie Sparkman, Ashley N. Sankey, Monique N. Westley, Eric J. Fontenot, Eric Stewart, Heba A. Sarhan, Fiyinfolu T. Mustapha, Thomas Vu, Briana M. Jarrett, Christian Clement, Partha S, Bhattacharjee, Deborah E. Sullivan, Cindy B. Morris and James M. Hill

Xavier University of Louisiana, LSU Health Science Center, Tulane University

Herpes simplex virus type 1 (HSV-1) infects greater than 90% of humans worldwide and during ocular infection produces inflammation and angiogenesis that can lead to blindness. In the United States, HSV infection is the leading cause of infection-induced blindness; nearly 40,000 new cases are reported and 300,000 cases are treated yearly. Cyclin-dependent kinases, mostly known for their involvement in the cell cycle and transcription, are involved in HSV transcription and replication. Cyclin-dependent kinase 9 (CDK9) and its downstream target, serine 2-phosphorylated RNA polymerase II, are essential in HSV-1 transcription. To date there is little literature on the role of CDKs in HSV infection of the eye or on the efficacy of CDK inhibitors in preventing HSV-1-associated ocular neovascularization and its consequences. We are testing the hypothesis that cyclin-dependent kinase inhibitors and shRNA against CDK9 decrease angiogenesis and angiogenic signaling pathways upregulated by angiogenic factors *in vivo* and *in vitro*. To date, we have demonstrated that these compounds decrease vascular endothelial cell migration, invasion, tubule formation *in vitro* and angiogenesis in chick embryo and mouse Matrigel angiogenesis models. Recently, we determined that the compounds tested are non-toxic in rabbit and mouse eyes, and both drugs decrease mouse corneal neovascularization due to HSV-1 infection to levels observed in mice treated with 1% trifluridine control.

13 Replica Exchange Molecular Dynamics on Cloud Computing Platform

Jin Niu, Ebrahim Khosravai and Shuju Bai
Southern University and A&M College

Cloud computing has emerged as a very powerful computing paradigm with the advantage of virtualization, scalability, fault tolerance, and a usage based pricing model. Replica Exchange Molecular Dynamics (REMD), as a scientific application, is a powerful technique which can improve the sampling of the potential energy during the process of Molecular Dy-

namics. Because of the high degree of independency of the individual simulation replicas, it is suitable to convert REMD into a cloud computing an application.

In our research, we implemented REMD using two MD simulation packages, Charmm and NAMD on traditional high performance computing clusters and cloud computing clusters. A series of experiments were designed to investigate the performance of REMD in HPC and cloud computing environments. Our experiments emphasized on the comparison of efficiency and effectiveness of computation and communication between cloud computing platform and parallel computing platform with identical hardware configurations. HPC cluster QueenBee of LONI was used as our HPC platform, and VCL of Southern University was the cloud computing platform. Hadoop framework was adopted to implement the REMD algorithm.

14 Phosphorylation of p65 NF-KappaB upon TNF treatment is regulated by DNA-dependent protein kinase

Samuel Okpechi, Youssef Errami, Mohammad Q. Abughazleh and Hamid Boulares
Southern University at New Orleans, LSUHSC - New Orleans

The objective of this study is to investigate whether DNA-PK regulates TNF-induced ICAM-1 expression in the colon cancer cell line HCT 116 and to identify the underlying mechanism of such regulation. Our hypothesis is that DNA-PK regulates TNF-induced ICAM-1 expression through phosphorylation of p65 NF-KappaB. Our lab has recently published that DNA-dependent protein kinase (DNA-PK) induces VCAM-1 expression upon TNF stimulation by phosphorylation of p50 NF-KappaB. To test our hypothesis, we utilized the human HCT116 colon cancer cell line (HCT-wt) and genetically modified versions of the cells that are heterozygous (HCT +/-) for DNA-PKcs. DNA-PKcs knockout cells were excluded from this study given the severe effect that it has on many aspects of cellular processes. Additionally, DNA-PK heterozygosity mimics drug treatments with DNA-PK inhibitors in clinical settings. Interestingly, treatment of HCT-wt cells with TNF did not induce expression of VCAM-1 despite the fact that DNA-PKcs heterozygosity severely reduced the ability of the cells to migrate in an in vitro migration assay performed earlier in the lab. These results led me to examine whether DNA-PK plays a role in ICAM-1 expression. TNF was the chosen cytokine treatment for it had the strongest effect on the HCT-wt group in comparison to IL-4 and LPS (endotoxin). DNA-PKcs heterozygosity severely reduced expression of ICAM-1 in response to TNF treatment. Such regulation appears to take place at the transcription levels as assessed by RT-PCR. DNA-PKcs deficiency severely reduced the ability of HCT116 cells to respond to TNF treatment as p65 NF-KappaB phosphorylation and nuclear translocation were compromised. Such effects may be linked to an effect on I-KappaBAlpha phosphorylation and degradation. These results suggest that DNA-PK plays an important role in the regulation of ICAM-1 and overall inflammation by regulating NF-KappaB signal transduction.

15 Involvement of p38 MAP kinase, but not ERK-1/2 (p42/44), in histamine-induced endothelial actin reorganization and barrier disruption.

Joseph Olubadewo, Jerome Breslin, Curtis Lawrence and Eyong Madonia
Southern University at New Orleans and University of South Florida

Our working hypothesis: histamine increases the endothelial hyperpermeability by activation of the p38 and p42/44 MAP kinases. Transendothelial electrical resistance (TER) of human umbilical vein endothelial cells (HUVEC) grown on small gold electrodes served as an index of barrier function, before and after addition of 10 μM histamine. The role of p38 MAPK was tested with 6 μM SB203580 and p42/44 with 10 μM PD98059 or 1 μM U0126. Specific, dual phosphorylation of p38 MAPK or p42/44 on their activation sites was detected by Western blotting. Dynamics of GFP-actin and VE-cadherin-GFP expressed in HUVEC were also evaluated. The results show that histamine increased phosphorylation of both p38 MAPK and p42/44, which was blocked by pretreatment with SB203580, or PD98059/U0126, respectively. Histamine-induced decreases in TER were inhibited by SB203580, but not affected by PD98059 or

U0126 pretreatment. Histamine did not change VE-cadherin-GFP organization, but did briefly stop GFP-actin-rich edge protrusions. However, these protrusions were not decreased by histamine when the cells were pretreated with SB203580. The data suggest that p38 MAPK, but not p42/44, mediates histamine-induced endothelial barrier disruption by reducing endothelial cell spreading motions.

Supported by NIH grants R01HL098215 and P20GM103424, NSF grant HRD0928797, and the Louisiana Board of Regents Support Fund LBRN 67515.

16 Development of high throughput screening model to investigate the effect of drugs on the blood brain barrier permeability

Hisham Qosa, Jeffrey Keller and Amal Kaddoumi
University of Louisiana at Monroe, Pennington Biomedical research center

Blood brain barrier (BBB) is a tight junction monolayer of cerebral capillary endothelial cells that physically separates the blood from brain. Although BBB regulates the levels of drugs inside the brain, it could be affected by many drugs that may change its permeability. Different models were developed to study the effect of drugs on the permeability of BBB. However available models are complex, expensive and they are not suitable for screening of large number of drugs. The aim of this study was to develop an in vitro model that is reliable, sensitive, and inexpensive to screen the effect of large number of drugs on the permeability of BBB. In this study, high throughput filtration plates composed from mixed cellulose ester (MCE) and 96-well polycarbonate HTS plate were used as a scaffold for growth of the mouse brain endothelial cells bEnd3. Cells were visualized by microscope after staining of cells, and permeability studies were performed. In addition, MTT assay, to determine cell viability, and lucifer yellow permeability were evaluated at different plating days. Results from these studies indicated that bEnd3 cells form a confluent monolayer on the MCE membrane filters and on the polycarbonate HTS plates. Cells viability increased after plating to reach plateau at day 5. Lucifer yellow permeability results were inconsistent with filter plates while they were more reliable and less variable with polycarbonate HTS plates. Further optimization and validation of polycarbonate HTS plate are currently in progress in our laboratory at ULM to improve the tightness of this model. Future studies will include investigation of drugs effect on the tightness and integrity of the cell monolayer.

17 Epigenetic regulation of α-actinin (1,4) by miRNAs in glioblastomas

Quincy Quick, Francesca Peruzzi
Southern University at New Orleans and LSUHSC - New Orleans

Brain tumors, specifically glioblastomas, have been unresponsive to traditional and contemporary clinical treatment regimens and protocols utilized to combat this disease. The refractoriness of brain tumors to preventative and curative care can be attributed in part to a high proliferative index and high rate of tumor recurrence of this human cancer. We recently demonstrated that isoforms of the actin-binding protein α-actinin (1,4), expressed at high levels in clinical and experimental specimens of glioblastomas, contribute independently to glioblastoma proliferation and migration. However to date little is known regarding the molecular mechanisms that underlie α-actinin (1,4) function. To this end we examined here the epigenetic regulation of α-actinin (1,4) by overexpressing miRNA 122 and 374 in glioblastoma cells. Overexpression studies revealed that miRNA-122 and miRNA-374 which target α-actinin 1 and 4, respectively, did not downregulate α-actinin (1,4) protein expression in glioblastoma cells as determined by immunofluorescence analysis. These data provide evidence that although α-actinin (1,4) are targets of miRNAs 122 and 374 as predicted by bioinformatic algorithms these small-inhibitory RNAs appear to have no regulatory function of α-actinin (1,4) in glioblastomas. Alternatively, we are evaluating miRNAs 153 and 218 for their regulatory functions of α-actinin 1 and 4 in glioblastomas as a number of miRNAs target this actin-binding protein with divergent cell behavior roles in glioblastomas.

18 Neuroprotectin D1 (NPD1), a Novel Bioactive Lipid, Upregulates Iduna Expression as a Cell Survival Signal in Retinal Pigment Epithelial (ARPE-19) Cells

Megan Richters, Dr. Pranab Mukherjee and Dr. Nicolas Bazan
University of Louisiana at Monroe

Iduna is a neuroprotective protein against glutamate NMDA receptor-mediated excitotoxicity both in vitro and in vivo through interfering with PAR polymer-induced cell death. Mutation at the PAR polymer binding site abolishes the PAR binding activity of Iduna and attenuates its protective actions. NPD1, a docosahexanoic acid-derived mediator, induced cell survival through the upregulation of Bcl2 class of survival proteins under oxidative stress (OS) in retinal pigment epithelial (RPE) cells. The goal of this study is to examine if NPD1 survival bioactivity engages Iduna expression in RPE cells undergoing OS. We used ARPE-19 cells for our study. 72h grown ARPE-19 cells were serum starved overnight, OS was introduced by H2O2, 600uM/TNF-alpha, 10ng/ml, and then challenged with NPD1 for 3, 4, 6, 8, 10, and 12h. Iduna protein was detected in Western blot analysis by using anti-Iduna antibody (RNF146) and apoptotic cell death was detected by Hoechst staining. Our results indicated that NPD1 at 50 and 100 nM concentrations enhanced expression of Iduna in ARPE-19 cells undergoing OS. However, OS or NPD1 alone did not have any effect on the Iduna expression. Transfection experiments with Iduna expression vectors showed that human and mouse Iduna expressions were upregulated by NPD1 in ARPE-19 cells under OS. On the other hand shRNA, which abolishes Iduna expression, and YRAA, which causes a mutation at the PAR binding site of Iduna, constructs were unable to induce Iduna expression by NPD1 under similar conditions. Moreover, the NPD1-mediated expression of Iduna attenuated apoptosis induced by OS in ARPE-19 cells. Finally, our observations identify that NPD1 exerted enhanced expression of Iduna in RPE cells undergoing OS. To our knowledge, this is the first report that the bioactive lipid molecule NPD1 mediates enhanced expression of Iduna in RPE cells. This NPD1 mediated expression of Iduna may be a key regulator of cell survival in OS mediated cell death.

19 Combining visualization with data mining for bioinformatics-driven discovery

Phoebe Rollyson and Trutschl Cvek
Louisiana State University - Shreveport

Life science is flooded with large and complex data. It is the job of computational tools developed for this arena to help uncover the patterns, forming hypotheses and addressing biomedical questions. On the other hand, it is the job of computational scientists to develop and utilize the tools to guide life scientists on their path to discovery.

We are showcasing some of the tools we utilize on a daily basis, spanning from open source to proprietary and commercial. We believe that it is important to utilize visualizations not only as static images but also as explorative tools. For this purpose, we designed a suite of algorithms that combine classic visualizations with neural networks called iNNfovis. These algorithms utilize a modified Kohonen's self-organizing map to visually cluster related records across multiple dimensions - we no longer have to choose which dimensions to drop in order to visualize them in a meaningful manner. Recently, we completed several studies using these tools, for example the study of gene expression patterns in the brain and spleen of mouse models for multiple sclerosis.

Supported by grants from the National Center for Research Resources (5P20RR018724-10) and the National Institute of General Medical Sciences (8 P20 GM103433-10) from the National Institutes of Health.

20 Identification of Fusarochromanone activity through DNA microarray analysis and qRT-PCR

Phoebe Rollyson, Trey King, Mickeal Key, John Clifford, Jennifer Roberts Gill, Yoon-Jee Kim, Elahe Mahdavian, Urska Cvek, Brian Salvatore, Brian Furmanski, Robert Rhoads and Tara Williams-Hart
Louisiana State University - Shreveport, LSUHSC - Shreveport, SIGA Technologies

Fusarochromanone is a toxic metabolite produced by fungi from natural grain or cereal plants. It was first recognized as a causal agent of various plant diseases and linked to bone deformation in poultry. Fusarochomanone, or FC101a, has been observed to increase apoptosis in tumor cells in vitro and reduce tumor growth in vivo. It has been found to inhibit cancer growth in a number of human cancer cell lines and has shown promise as a viable consideration for cancer therapy.

Identification of those genes that are differentially expressed as a result of FC101 treatment will lead to detection of the cellular mechanisms that interact with the drug to inhibit the growth of cancer cells. In an attempt to identify these molecular targets, DNA microarray analysis was performed on both human bladder cancer cells (UM-UC14) and Saccharomyces cerevisiae (budding yeast). Among human cancer cells tested in this study, bladder cancer cells were found to be more sensitive to the effects of FC101a and S.cerevisiae is widely used as a model organism in genetic studies. It is a simple, yet well-defined eukaryotic genetic system whose cellular activities are very similar to our own. It can be easily cultured, grows rapidly, and can be handled with minimal precautions.

DNA microarray analysis revealed a number of orthologous yeast and human genes that exhibit differential expression greater than two-fold when treated with FC101a. Subsequent Ingenuity Pathway Analysis revealed that a number of the differentially expressed genes are involved in apoptosis, cell cycle regulation, chromatin remodeling, and ribosomal assembly pathways. qRT-PCR is being performed on a select number of these genes in order to verify these findings.

21 Rank based non linear correlation for the analysis miRNA expression

Arun Prasath Shanmugam
Louisiana Tech University

Nuclear factor (NF-κB) is considered as one of the most pro-inflammatory signaling pathway in Alzheimer's disease (AD), based on associated pro-inflammatory genes including cytokines, chemokines, and adhesion molecules. Recent studies postulate that specific microRNAs (miRNA) are part of the regulatory mechanism of the inflammatory processes in AD. It is known that at least 4 miRNAs namely miRNA-9, miRNA-125b, miRNA-146a and miRNA-155 are responsible for the pathogenic mechanism of complement factor H (CFH) that leads to inflammatory neuro-degeneration. In this study our objective is to identify related miRNAs from a data set consisting of 1922 miRNAs that are functionally correlated to the above four miRNAs. However this poses the computational challenge of handling a large number of genes with less number of samples (features), traditionally known as the small n large P problem. To address this challenge we propose a novel ranked based non-linear correlation technique to capture similarities between miRNAs using the Bartels test. We hypothesize that this non-linear correlation technique can effectively cluster miRNA to functional significant groups, when the features are less. The aim of the proposed feature ranking technique using the ranked version of von Neumann's ratio (RVN) is capable of selecting functionally significant miRNAs, that is validated using the unsupervised hierarchical clustering. We compare our results with traditional Euclidean and Pearson correlation measures of similarity.

22 Effect of Various Transformations on the Responsive Distribution and on Gene Selection on Gene Expression Data for Gastric Cancer

Kankana Shukla, Dr. Prerna Dua, Dr. Hilary W. Thompson
Louisiana Tech University, LSUHSC - New Orleans

Gastric cancer has been linked to various factors but in spite of intensive research, its fundamental cause remains unknown. Most patients of gastric cancer are asymptomatic, making the diagnosis of the disease challenging. Gene expression analysis techniques have clinically proved to improve diagnosis and prognosis of diseases. Thus gene expression analysis has a high potential to considerably contribute in the early detection of the stomach cancer. One of the common challenges in analyzing DNA microarray data is the curse of dimensionality. Additionally, the presence of noise and complex biological interactions in a gene network further complicates the analysis of data expression data. Thus, for accurate classification of the disease, discovery of a subset of genes that contrast samples is crucial for understanding the underlying carcinogenic process.

In this work, we analyzed a previously never used gene expression data obtained from 569 adults (361 African Americans and 208 Caucasians) undergoing gastric biopsies for genomic DNA extraction. We undertook linear, logarithmic and cubic-spline preprocessing techniques for data transformation and compared their performances by constructing the corresponding classifier models. We also performed successive feature selection after transformation by the well-known feature selection techniques. We subsequently performed the supervised learning approach proving that the subsets of genes after transformation and feature selection produce better classifier models than the raw data model thus helping in the better classification for gastric cancer. We also came across seven genes that came up in our analysis that are proved to be responsible for gastric cancer in previous researches, validating our computational approaches. Our future work consists of performing pathway analysis on this data and obtain the biological validation.

23 A Comparative Analysis of Differential Expression, Feature Selection and Classification on miRNA Data for Alzheimer's Disease

Kankana Shukla, Dr. Prerna Dua and Dr. Walter J. Lukiw
Louisiana Tech University, LSUHSC - New Orleans

Alzheimer's is a complex neurological disease whose cause is still unknown. Several attempts have been made in the past to relate the miRNA regulation with the human diseases including Alzheimer's and many of those have proved to be significantly useful. In the current, research we investigate a miRNA microarray dataset that is obtained from three distinct regions (hippocampus, temporal lobe and control) of human brain from fifteen Alzheimer's disease patients over a period of 10 years and has an account of 1922 miRNAs. In this study, we scrutinize the four miRNA: hsa-miR-125b-5p, hsa-miR-9-5p, hsa-miR-146a-5p and hsa-miR-155-5p which have previously been stated to be cooperatively or redundantly regulating an inflammation pathway for Alzheimer's and thus playing a subtle role in the disease. In an attempt to find other miRNAs that possess a pattern similar to these four miRNAs, we performed various computational techniques on the data including- differential expression analysis, feature selection and difference in averages, that provided us with subsets of ranked miRNA based upon their significance. Subsequently, we came up with 21 additional miRNAs that show a similar pattern to the four referenced miRNAs. We performed a biomedical literature search on these newly discovered miRNAs to validate the results.

24 Seeking the Cause of Multiple Sclerosis by Screening Associated Genomic Regions for Diverged Sequence Homology

Jeffry Shultz
Louisiana Tech University

Multiple sclerosis (MS) is a disease in which oligodendrocytes, cells that insulate signals carried along neural axons, are destroyed, slowing or stopping nerve signals. Multiple genomic regions and environmental factors have been linked to MS, but the primary question has not been answered. Why are oligodendrocytes destroyed? Using a heuristic algorithm, I seek to identify potentially highly diverged sequences recognized by the human immune system as pathogenic, resulting in cell destruction. There are three related mechanisms to be tested. Mechanism 1. Alternative splicing/rare transcription of genes from MS associated chromosomal regions lead to similar protein domains that trigger an autoimmune response. Mechanism 2. Alternative splicing/rare transcription creates a protein similar to a pathogen previously recognized by the host immune system, causing the immune system to identify and destroy the oligodendrocyte as a pathogen. Mechanism 3. Alternative splicing/rare transcription creates multiple protein domains, each of which is similar to a sequence from a different pathogen that is recognized by the immune system and causes the immune system to identify and destroy the oligodendrocyte as a pathogen. A total of 158 human subject DNA have been collected, of which 24 are MS positive and 134 are MS negative. All three mechanisms have been tested and have produced promising preliminary sequence segregation between MS subject classes.

25 Hyperthermia induced by Gold Nanoshells and its effect on Lymphatic Endothelial Cells

Israel Soto, Merilyn Jennings, W. Todd Monroe, J. Steven Alexander and Kui Chen
Louisiana State University - Shreveport, Louisiana State University - Baton Rouge, LSU Health Science Center - Shreveport

In an attempt to control tumor metastasis through the lymphatic system, Gold Nanoshells (AuNS) are being used to induce hyperthermia in lymphatic endothelial cells (LECs) in order to inhibit the growth of lymphatic vessels from the targeted tumor to adjacent tissue. The induction of hyperthermia in the LECs is done by exploiting the unique photothermal properties of the AuNS. The AuNS are able to produce heat when exposed to light of a certain wavelength. The temperature profiles of the AuNS have been studied with the aid of a thermocouple and thermal camera. Currently tests are being conducted in which LECs are incubated with AuNS and then exposed to a light source. A lactase dehydrogenase assay is being used in order to determine cell viability after cells are exposed to the AuNS and light source.

26 Changes in plasma and renal tissue levels of various Inflammatory Cytokines in mice lacking the endothelial isoform of nitric oxide synthase during high salt intake

Roxan Stephenson, Dr. Dewan SA Majid and Dr. Purnima Singh
Grambling State University

Salt loading in humans and experimental animals causes progressive increases in blood pressure and inflammation, leading to endothelial dysfunction and renal damage. It has been demonstrated that a deficiency in the production of nitric oxide is linked to salt sensitivity and hypertension. Recent studies revealed that infusion of L-Nitro-Arginine-Methyl Ester (L-NAME) to anaesthetized mice, leads to decreased production of anti-inflammatory cytokine (Interleukin-10, IL10) and increased production of pro-inflammatory cytokine (Tumor Necrosis Factor-alpha, TNF-α).These cytokines are considered to be involved in the development of salt sensitive hypertension. In the present study we hypothesize that the inhibition of endothelial isoform of nitric oxide synthase leads to the reduced production of IL-10 and increased production of pro- inflammatory cytokines TNF-α, IL-6, IL-1β, MCP-1 during salt-sensitive hypertension. This study was conducted in male

mice, ≈ 7-8 weeks old, C57BL6 (WT) mice and B6.129P2-Nos3 (eNOS-KO). These were grouped as Normal Salt (NS) and High Salt treated (HS, containing 4% high Na for 14 days). Tail-cuff method was used to measure systemic blood pressure. Urine samples were analyzed for flow rate, and sodium and potassium concentrations. Plasma and renal tissues were collected to analyze various cytokine levels using ELISA kits. HS intake increased the MAP in eNOS-KO and increased urine flow and sodium excretion in both groups. HS intake had no effect on plasma and renal IL-10 but increased the plasma levels of TNF-α in both groups after HS. NS treated eNOS-KO had higher plasma IL-6 levels which was reduced after HS. HS treatment increased the plasma level of IL-1β in WT. HS intake reduced the renal MCP-1 level in both WT and eNOS-KO. The inhibition of endothelial isoform of nitric oxide synthase leads to increased plasma levels of TNF-α in both WT and eNOS-KO after HS treatment but decreased plasma and renal IL-6 levels in eNOS-KO.

27 Role of Chromatin Insulator Elements in HSV-1 During Latency and Reactivation

Donique Thorpe, Monica Ertel, Amy Cammarata and Dr. Donna Neumann, Ph.D
Dillard University and LSUHSC - New Orleans

The Herpes Simplex Virus-1 is capable of establishing latency inside its host. During the latent stages of the infection only the latency associated transcript, the LAT gene is expressed while the remainder of the genome is repressed by epigenetic mechanisms. This organization indicates the presence of functional insulator elements in HSV-1. Recent research has subsequently shown that the LAT gene is flanked by a CTCF binding motif (annotated B2), that has been characterized as an insulator with enhancer blocker and silencer activities. In addition to this CTCF binding motif, there are six other CTCF binding motifs in the HSV-1 genome, none of which have been characterized as insulators to date. The objective of this research project is to determine whether the remaining six CTCF binding motifs of HSV-1 are capable of insulator functions, such as enhancer blocking and/or silencing functions. To meet the goals of our objective, we have designed and constructed several reporter plasmid constructs containing the core CTCF binding domains and ~500 bp of HSV-1 sequence flanking each domain to assess the ability of each CTCF binding domain to act as an enhancer blocker to the LAT. To test the silencing ability of each core CTCF domain, additional constructs were generated with ~100 bp deletions of only the reiterated CTCF binding sequences. These reporter constructs were transfected into rabbit skin cells and the luciferase activity measured in a dual reporter assay. Our results show that at least one other CTCF binding motif (annotated B6) acts as an insulator to the LAT during latency in HSV-1. Future research includes developing recombinant DNA constructs which lack the core repeats then phenotype it into rabbits to determine changes in viral pathology. "LBRN projects were supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103424 and by the Louisiana Board of Regents Support Fund.

28 An unsupervised learning approach using statistical technique Median-centered Expression Data set to effectively identify clusters of miRNAs in Alzheimer's disease.

Ashwini Weber, Dr. Prerna Dua, Dr. Walter J.Lukiw and Dr. Pradeep Chowriappa

Louisiana Tech Univeristy

The standard statistical method opted for omics studies to identify biomarkers using the difference in mean levels of expression levels, recent research postulate that the rate of detection of significant miRNAs increases when using differential variability (DV) in gene expression levels. DV is a statistical technique that is used to characterize gene expression based on significant increase or decrease in the variability of the gene expression. For our analysis, we use microarray samples to extract miRNAs from three regions of the brain: the Control (whole brain), the Temporal Lobe, and the Hippocampus. The objective of this work is to provide a comparative analysis between traditional standardization techniques such as Z-score,

and Min-Max standardizations and the proposed technique based on Outlier Robust T-statistics (ORT). The proposed technique is believed to effectively capture the differentially variable miRNAs using the Median-centered Expression Data set (MED) analysis. For our experimental setup all the 15 samples are considered for the analysis - Control (5), Temporal Lobe (5), Hippocampus (5) with 1922 genes each. We hypothesize that ORT replaces sample mean by sample median to achieve a robust variance estimate for small number of samples.As a comparison of effectiveness of ORT, we applied Z-score and Min-Max to the same miRNA dataset. We then subject resultant normalized miRNA data from each method to principle component analysis to characterize those miRNAs that are up and down regulated. An unsupervised learning technique (K means clustering) is intended to be applied on the miRNA dataset. We propose that ORT will efficiently provide clusters of miRNAs that behave in similar fashion compared to other standardization techniques. This will give us an insight on the effect of miRNAs on the disease and their biological significance.

Notes

Notes

Oral Presentation Abstracts

8:15 - 8:35 am

Teresa A. Murray[1], Andrew A. George[2], Paul Whiteaker[2], Annadora Bruce-Keller[3], Yuri Voziyanov[1]

[1] **Louisiana Tech University**, Ruston, LA; [2] Barrow Neurological Institute, Phoenix, AZ; [3] Pennington Biomedical Research Institute, Baton Rouge, LA

A recently discovered cholinergic neurotransmitter receptor subtype, the α7β2 nicotinic acetylcholine receptor (α7β2-nAChR), is expressed in neurons in the septum and hippocampus of the rodent brain. These regions experience a loss of neurons expressing cholinergic receptors in Alzheimer's disease (AD), in humans and in rodent AD models. This loss contributes to the decline in cognition and memory as the disease progresses. Intracellular deposits of amyloid-β1-42 peptide (Aβ1-42) have been identified as an early step in AD etiopathology. Notably, α7-nAChR-mediated endocytosis of Aβ1-42 peptide results in intracellular deposits of Aβ1-42in AD. The α7β2-nAChR is similar in functional attributes to the well-recognized α7-nAChR subtype. This researcher has previously constructed a model of the level of α7β2-nAChR function based on the number and positions of the α7 and β2 subunits within this pentameric receptor. Similarly, stoichiometry and position may affect the rate of internalization. However, the relative potential for α7β2-nAChR-mediated endocytosis by this newly discovered receptor has not been studied. To investigate the effect of stoichiometry and position on Aβ1-42 internalization, a set of concatemeric cDNA constructs was created and tested to ensure that they indeed express as functional receptors. The cDNA will be used to transfect native nAChR-null SH-EP1 cells which will then be incubated with Aβ1-42, or Aβ1-42 scrambled peptide, to quantify the volume and intracellular position of peptide deposits, as well as cell viability as it relates to deposit volume and/or localization. In order to show potential colocalization of Aβ1-42 with internalized receptors separate subunit constructs, each fused to its own color of fluorescent protein, have been expressed in SH-EP1 cells. Lines have been created that express the canonical α7-nAChR, the new α7β2-nAChR and one expressing only the β2-nAChR subunit. Aβ1-42 will be stained for quantification and colocalization analyses based on 3D reconstructions of confocal microscope images.

8:35 - 8:55 am

Characterization of Hereditary Spastic Paraplegia-Causing Mutations in Kinesin Kif5A

Thomas Huckaba, Edward Wojcik and Sunyoung Kim

Xavier University of Louisiana and LSUHSC-New Orleans

Hereditary Spastic Paraplegias (HSPs) are a group of neurodegenerative disorders that arise from the progressive degeneration of corticospinal tract axons, causing lower limb spasticity and weakness. An autosomal dominant form of HSP (AD-HSP) is caused by mutations in Kif5A, a neuronally enriched form of the kinesin-1 family of cellular transport motors. While nineteen separate AD-HSP-causing missense mutations in Kif5A have been mapped, eighteen are in the motor domain, suggesting a deficit in catalytic activity as the mechanistic cause of the disease. We have mutated the wild type Kif5A gene with each of the separate AD-HSP-causing mutations and have begun to test the mechanical properties of these recombinantly-expressed mutant motors in a series of in vitro biochemical and biophysical assays. Here we report the results of four separate mutations in the ATP binding and hydrolysis pocket (S202N, S203C, R204W, and V231L) and one mutation in the L11 loop predicted to be at the microtubule interface (E251K). Performing microtubule pelleting assays in the presence of saturating levels of ATP, we found that S202N and S203C had a significantly higher microtubule affinity than wild type Kif5A or the other mutants. Conversely, in the presence of the non-hydrolyzable ATP analog AMPPNP, R204W, V231L and E251K had a significantly lower microtubule affinity than wild type and the other mutants. In microtubule gliding assays, wild type Kif5A moved microtubules at a rate of 0.47 ± 0.02 μm per second. The S203C mutant moved microtubules at a 60-fold slower rate of 0.0074 ± 0.0028 μm per second, while the S202N, R204W, and E251K mutants bound microtubules to the glass surface in rigor and the V231L mutant was unable to recruit microtubules from solution. These contrasting results suggest that AD-HSP may be caused by a variety of different mechanical deficits in the kinesin catalytic core.

Oral Presentation Abstracts

8:55 - 9:15 am

Targeting Beta Amyloid Clearance as Therapeutic Approach for Alzheimer's Related Disorders

Amal Kaddoumi and Dr. Jeff Keller
University of Louisiana at Monroe and PBRC

Blood-brain barrier (BBB) dysfunction has a critical role in Alzheimer's disease (AD) and can promote the accumulation of b-amyloid (Ab) in cerebral blood vessels, and in many cases lead to the initiation of cerebral amyloid angiopathy (CAA), which present in 80% of AD cases. While exact causes for BBB dysfunction in AD and CAA are not well known, faulty Ab clearance across the BBB has been suggested as a possible contributing factor to both disorders, based on Ab clearance having a key role in determining brain Ab levels. Recent studies support a role of P-glycoprotein (P-gp) in Ab clearance from the brain across the BBB, which is down-regulated with age and in AD. The mechanism, specificity, and extent of P-gp contribution to the deposition and toxicity of Ab remain unclear. Our preliminary data from in vitro uptake and transport studies, in addition to in vivo Ab clearance studies across the BBB of wild type mice, have demonstrated P-gp has essential role in Ab clearance, and its expression at the BBB is important to regulate brain Ab levels. The long-term goal of this project is to advance our understanding in the mechanism(s) of Ab elimination across the BBB so that advances in diagnostics, and new interventions to prevent or delay the onset of Ab pathology can be developed. The initial hypothesis of this work is that P-gp plays a specific role in the clearance of Ab across the endothelial cells within the BBB. Data from Specific Aim 2 will be presented, demonstrating the association between P-gp and Ab using Ab efflux kinetic assays. Based on these new results, we have expanded our original hypothesis, now testing the hypothesis that "enhanced clearance of Ab by cerebrovascular endothelial cells will be beneficial to BBB homeostasis and prevent the development of CAA". Key to these new efforts is the development of a BBB model applicable for high-throughput screening of compounds that restore BBB function following Ab exposure, and reduce Ab toxicity.

9:15 - 9:30 am

Epigenetic regulation of α-actinin (1,4) by miRNAs in glioblastomas

Quincy Quick and Francesca Peruzzi
Southern University at New Orleans and LSUHSC-New Orleans

Brain tumors, specifically glioblastomas, have been unresponsive to traditional and contemporary clinical treatment regimens and protocols utilized to combat this disease. The refractoriness of brain tumors to preventative and curative care can be attributed in part to a high proliferative index and high rate of tumor recurrence of this human cancer. We recently demonstrated that isoforms of the actin-binding protein α-actinin (1,4), expressed at high levels in clinical and experimental specimens of glioblastomas, contribute independently to glioblastoma proliferation and migration. However to date little is known regarding the molecular mechanisms that underlie α-actinin (1,4) function. To this end we examined here the epigenetic regulation of α-actinin (1,4) by overexpressing miRNA 122 and 374 in glioblastoma cells. Overexpression studies revealed that miRNA-122 and miRNA-374 which target α-actinin 1 and 4, respectively, did not downregulate α-actinin (1,4) protein expression in glioblastoma cells as determined by immunofluorescence analysis. These data provide evidence that although α-actinin (1,4) are targets of miRNAs 122 and 374 as predicted by bioinformatic algorithms these small-inhibitory RNAs appear to have no regulatory function of α-actinin (1,4) in glioblastomas. Alternatively, we are evaluating miRNAs 153 and 218 for their regulatory functions of α-actinin 1 and 4 in glioblastomas as a number of miRNAs target this actin-binding protein with divergent cell behavior roles in glioblastomas.

9:30 - 9:45 am

Role of inosine hydrolases in Bacillus anthracis spore germination

Rebecca Giorno-McConnell

Louisiana Tech University

Bacillus anthracis spores are the infective particle of the disease anthrax. Our goal is to understand the role of the exosporium in germination and dormancy. Although dormant, spores can resume metabolic activity in the presence of nutrients. This process, called germination, results in the shedding of the outer spore protective structures (the exosporium and the coat) and the return to vegetative growth. In Bacillus thuringiensis, germination in response to inosine can be controlled by the abundance of a spore-specific inosine hydrolase (IunH). We are studying the effects of inosine hydrolases, annotated as iunH and bas3345, on germination and spore dormancy. To investigate the hypothesis that abundance of either inosine hydrolase influences a spore's ability to respond to the germinant inosine, we are making a number of mutant strains. Previously, we showed iunH mutant spores germinate more completely based on a greater percent final loss of optical density (OD) in limiting levels of inosine with various amino acids. In this study, we quantitated the conversion of spores from dormant which appear phase bright to germinated which appear phase dark in the presence of excess inosine (1 mM) and alanine (0.5 mM). The percent phase dark after 45 minutes for wild type and the iunH mutant are 83.2% and 94.5%, respectively, suggesting that iunH mutant spores germinate more completely even in abundant nutrient. These data suggest IunH influences germination by degrading inosine. This in turn allows persistence and/or survival of B. anthracis in the environment or during infection by preventing spores from germinating when there is limited nutrition. Future studies will investigate IunH inhibitors to see if they influence germination in wild type spores which may aid in the development of better therapeutic and decontamination strategies.

9:45 - 10:00 am

Point-of-care Microelectronic Diagnostics For Early Phase Rickettsial Infections

Adarsh Radadia, Kevin Macaluso, Long Que, Wenli Zhang and Scott Li

Louisiana Tech University and LSU-BR

Miniaturized point-of-care diagnostics that detect Rickettsia in serum at clinically relevant concentrations can help overcome time and labor needs of current methods and improve patient survival rates. Overall goal of this project is to develop a portable microfluidic biosensor that concentrates R. parkeri from serum using dielectrophoresis and antibody decorated electrodes, and electrically detects such concentration by impedance spectroscopy. This LBRN project tested two hypotheses in developing this biosensor. Our first hypothesis, existence of dielectrophoretic settings for maximum R. parkeri capture, was tested by flowing serum containing heat-killed R. parkeri (10 - 10^7 cells/ml) through the sensor while varying the dielectrophoretic voltage, frequency and sample flow rate. Microscopic observation of dielectrophoretic concentration was observed by fluorescently labeling R. parkeri. We found that conditions for dielectrophoretic concentration of R. parkeri resulted in electrolysis of undiluted or diluted serum, quite contrary to our experience with E. coli. This led us to develop an alternative approach using immunolatex beads for capture of R. parkeri from serum and their resuspension in isotonic media for dielectrophoretic concentration. Our second hypothesis, impedance biosensing of Rickettsia and its augmentation using dielectrophoresis, was tested by flowing serum containing heat-killed R. parkeri (10 - 10^5 cells/ml) through the sensor with and without dielectrophoresis. We detected distinct impedance changes for R. parkeri dilutions down to 10^3 cells/ml without dielectrophoresis. While we could not apply dielectrophoresis in serum, our future work will use dielectrophoretic labels such as immunolatex beads to enable concentration of Rickettsia and lowering detection limit to 10 cells/ml.

10:15 - 10:35 am BREAK

10:35 - 10:55 am

Assessment of the molecular target of fusarochromanone

Tara Williams-Hart, Phoebe Rollyson, Trey King, Mickeal Key, John Clifford, Jennifer Roberts Gill, Yoonjee Kim, Elahe Mahdavian, Urska Cvek, Marjan Trutschl, Brian Salvatore, Brian Furmanski and Robert Rhoads

LSU-Shreveport, LSU Health Sciences Center and Siga Technologies

Several investigations identify fusarochromanone (FC101) as a potential, novel anti-cancer compound. These studies revealed that FC101 inhibits angiogenesis, inhibits growth of 35 human cancer cell lines, increases apoptosis of melanoma cell lines, and significantly reduces tumor growth in mice. The long-term goal of this project is to elucidate how FC101 inhibits cancer cell growth by identifying its molecular target(s). In this study, we used DNA microarray analysis and qRT-PCR to identify genes that are differentially expressed in response to FC101 treatment in human bladder cancer cells and Saccharomyces cerevisiae, budding yeast. We hypothesize that changes in gene expression in response to FC101 treatment will elucidate cellular mechanisms by which Several investigations identify fusarochromanone (FC101) as a potential, novel anti-cancer compound. These studies revealed that FC101 inhibits angiogenesis, inhibits growth of 35 human cancer cell lines, increases apoptosis of melanoma cell lines, and significantly reduces tumor growth in mice. The long-term goal of this project is to elucidate how FC101 inhibits cancer cell growth by identifying its molecular target(s). In this study, we used DNA microarray analysis and qRT-PCR to identify genes that are differentially expressed in response to FC101 treatment in human bladder cancer cells and Saccharomyces cerevisiae, budding yeast. We hypothesize that changes in gene expression in response to FC101 treatment will elucidate cellular mechanisms by which FC101 inhibits cancer cell growth. Human bladder carcinoma cells (UM-UC14) were treated with FC101 for 24, 48 and 72 hours and budding yeast cells were treated with FC101 for 20 minutes. Subsequently, RNA was isolated and DNA microarray analysis was performed. Over 500 genes were identified as highly expressed and greater than two-fold up regulated or down regulated in FC101-treated cells compared with untreated cells using Ingenuity Pathway Analysis and Genesifter software. Of these FC101-responsive genes, 20 human and 20 yeast genes were verified by qRT-PCR. These results suggest that FC101 treatment may inhibit cancer cell growth by activating or inhibiting multiple cellular pathways, primarily apoptosis, cell cycle regulation, ribosomal assembly and chromatin remodeling.

10:55 - 11:15 am

Regulation of Mouse ES Cell Differentiation into Neurons by Hoxa1

Eduardo Martinez-Ceballos and Konstantin G. Kousoulas

Southern University and A&M College and LSU School of Veterinary Medicine

Vitamin A and its retinoid derivatives, such as retinoic acid (RA), play important roles during embryonic development and the maintenance of epithelial tissues in adults. Furthermore, vitamin A deficiency (VAD) is the most common cause of blindness in developing countries. While in adult animals VAD has been shown to affect spatial learning and memory impairment, during mammalian development the major target tissues of vitamin A deficiency include the heart, central nervous system, and the development of skull, skeleton and limbs, among others. These abnormalities are also evident from mice lacking functional retinoic acid receptors (RARs) or RAR target genes such as Hoxa1. Hoxa1 is a member of the homeobox (Hox) family of transcription factors and its importance during development is evident from the phenotype of the Hoxa1 knockout mice; they include deficiencies in skull development and in hindbrain development. In line with these observations, we have previously demonstrated that Hoxa1 is required for the neuronal differentiation of mouse embryon-

ic stem (ES) cells in culture, and have identified putative Hoxa1 target genes that play an important role during mouse neurogenesis. One of such targets, Neuronatin (Nnat), was found to possess putative Hoxa1-binding sites as determined by DNAse I hypersensitivity assays. Using a GFP reporter, we present here our preliminary steps toward the characterization of one of such putative sites which is located ~5.4 kbs upstream of the Nnat transcriptional start site. As HOXA1 mutations in humans have been associated with neurological disorders that arise from deficiencies in interneuronal function, understanding the mechanism of Hoxa1 action has implications in both basic research and in the development of future therapeutic applications.

11:15 - 11:30 am

Inhibition of HSV-1-associated ocular neovascularization by antiangiogenic agents

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Xavier University of Louisiana, LSUHSC-New Orleans and Tulane University

Herpes simplex virus type 1 (HSV-1) infects greater than 90% of humans worldwide and during ocular infection produces inflammation and angiogenesis that can lead to blindness. In the United States, HSV infection is the leading cause of infection-induced blindness; nearly 40,000 new cases are reported and 300,000 cases are treated yearly. Cyclin-dependent kinases, mostly known for their involvement in the cell cycle and transcription, are involved in HSV transcription and replication. Cyclin-dependent kinase 9 (CDK9) and its downstream target, serine 2-phosphorylated RNA polymerase II, are essential in HSV-1 transcription and replication however, to date there is little literature on the role of CDKs in HSV infection of the eye or on the efficacy of CDK inhibitors in preventing HSV-1-associated ocular neovascularization and its consequences. We are testing the hypothesis that cyclin-dependent kinase inhibitors and shRNA against CDK9 decrease angiogenesis and angiogenic signaling pathways up-regulated by angiogenic factors *in vitro* and *in vivo*. To date, we have demonstrated that these inhibitors decrease vascular endothelial cell migration, invasion, tubule formation *in vitro* and angiogenesis in chick embryo and mouse Matrigel angiogenesis models. Recently, we determined that the compounds tested are non-toxic in rabbit and mouse eyes, and both drugs decrease mouse corneal neovascularization due to HSV-1 corneal infection to levels observed in mice treated with an anti-herpetic control drug. We have begun to develop novel inhibitors of CDKs and will determine whether these compounds inhibit CDK9 *in vitro* and reduce corneal neovascularization due to recurrent HSV-1 infection *in vivo*.

11:30 - 11:45 am

Involvement of p38 MAP kinase, but not ERK-1/2 (p42/44), in histamine-induced endothelial actin reorganization and barrier disruption.

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Our working hypothesis: histamine increases the endothelial hyperpermeability by activation of the p38 and p42/44 MAP kinases. Transendothelial electrical resistance (TER) of human umbilical vein endothelial cells (HUVEC) grown on small gold electrodes served as an index of barrier function, before and after addition of 10 μM histamine. The role of p38 MAPK was tested with 6 μM SB203580 and p42/44 with 10 μM PD98059 or 1 μM U0126. Specific, dual phosphorylation of p38 MAPK or p42/44 on their activation sites was detected by Western blotting. Dynamics of GFP-actin and VE-cadherin-GFP expressed in HUVEC were also evaluated. The results show that histamine increased phosphorylation of both p38 MAPK

and p42/44, which was blocked by pretreatment with SB203580, or PD98059/U0126, respectively. Histamine-induced decreases in TER were inhibited by SB203580, but not affected by PD98059 or

11:45 - 12:00 pm

Photothermal injury to lymphatic endothelial cells by Au-nanoshell-mediated hyperthermia

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LSU-Shreveport, LSUHSC-Shreveport and LSU-BR

Recent studies suggested that tumor-associated lymphatics and lymphangiogenesis play important roles in promoting tumor growth and metastasis in many types of cancer. However, lymphatic system has received much less attention as a target of intervention in cancer treatment compared to the blood vascular system. We investigate the use of gold-nanoshell-mediated hyperthermia targeting lymphatic endothelial cells as a minimally invasive strategy for inhibiting tumor metastasis. Au nanoshells (AuNSs) are tunable core/shell nanoparticles that can be fabricated to strongly absorb in the near Infrared region and convert absorbed light to heat with high efficiency. A therapeutic dose of heat can be generated upon laser irradiation and induces photothermal injury to lymphatic endothelial cells (LECs). The working hypothesis is tumor metastasis will be suppressed when photothermal injury to lymphatic endothelial cells disrupts tumor-associated lymphatic vessels and inhibits lymphangiogenesis.

AuNSs of different sizes and aspect ratios were synthesized and their photothermal heating properties were characterized in vitro by measuring the temperature change upon laser irradiation. The effects of laser wavelength, laser power and AuNS concentration on the photothermal heating were examined. The kinetics and efficacy of AuNS-mediated hyperthermia for inducing photothermal injury to LECs was investigated in cell culture using the SV-LEC cell line. The LECs were incubated with AuNSs to study the uptake of AuNSs by LECs. Cytotoxicity of AuNSs and the cell viability after laser irradiation was evaluated using LDH assay based on the measurement of lactate dyhydrogenase release from cells. Future studies include the optimization of the parameters for inducing maximal photothermal injury and targeted delivery of AuNSs to LECs through surface functionalization.

12:00 - 12:15 pm

Development of high throughput screening model to investigate the effect of drugs on the blood brain barrier permeability

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University of Louisiana at Monroe and Pennington Biomedical research center

Blood brain barrier (BBB) is a tight junction monolayer of cerebral capillary endothelial cells that physically separates the blood from brain. Although BBB regulates the levels of drugs inside the brain, it could be affected by many drugs that may change its permeability. Different models were developed to study the effect of drugs on the permeability of BBB. However available models are complex, expensive and they are not suitable for screening of large number of drugs. The aim of this study was to develop an in vitro model that is reliable, sensitive, and inexpensive to screen the effect of large number of drugs on the permeability of BBB. In this study, high throughput filtration plates composed from mixed cellulose ester (MCE) and 96-well polycarbonate HTS plate were used as a scaffold for growth of the mouse brain endothelial cells bEnd3. Cells were visualized by microscope after staining of cells, and permeability studies were performed. In addition, MTT assay, to determine cell viability, and lucifer yellow permeability were evaluated at different plating days. Results from these studies indicated that bEnd3 cells form a confluent monolayer on the MCE membrane filters and on the polycarbonate HTS plates. Cells viability increased after plating to reach plateau at day 5. Lucifer yellow permeability results were inconsistent with filter plates while they were more reliable and less variable with polycarbonate HTS plates. Further optimization and validation of polycarbonate HTS plate are currently in progress in our laboratory at ULM to improve the

tightness of this model. Future studies will include investigation of drugs effect on the tightness and integrity of the cell monolayer.

12:15 - 1:30 pm LUNCH

1:30 - 1:50 pm

Evaluation of Fusarochromanone: A Potent Inhibitor of Angiogenesis and Tumorigenesis

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FC101 is a small molecule fungal metabolite that has very potent anti-cancer and anti-angiogenic activity. In this study we tested the hypothesis that FC101's molecular targets could be regulators of angiogenesis, signal transduction, cell proliferation, and/or programmed cell death (apoptosis). The detailed effects of FC101 on these processes were determined for a panel of human tumor cell types (SCC, breast, prostate, and bladder). Our data shows that FC101 has the potential to be an effective cancer therapeutic drug, because it suppresses both angiogenesis and tumorigenesis. Its broad-spectrum inhibitory effect on cancer cell lines and its direct inhibition of endothelial cell growth, suggest that it could be beneficial for treating a variety of human cancers. Scientists have traditionally turned to nature to find new lead compounds for fighting disease. Fungus and molds have produced many important new lead compounds for drug development. That is how FC101 was discovered, and it represents an important new lead compound for the treatment of cancer. However, the precise biological targets of FC101 remain unknown. Currently, we are using computer-based assays, photoaffinity labeling, and avidin-biotin affinity chromatography, to predict the FC101's mechanism of action and to synthesize more biologically active analogs. The chemical synthesis of novel analogs, as well as cell-based assays that probe specific signaling pathways plays a significant role in our current development of FC101 for cancer therapy.

1:50 - 2:10 pm

A study of differential expression of MiRNAs expression patterns discovered for Alzheimer's disease

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The superior temporal lobe neocortex and hippocampus of Alzheimer's disease (AD) patients show signs of significant changes in physiological function that are the hallmarks of AD. MiRNA networks control a substantial portion of the post-transcriptional gene regulation and hence an alteration in the expression of miRNAs is emerging as a significant contributing factor to AD. It is imperative to discover the biologically significant correlations among co-regulated miRNAs that play a substantial role in the progression of AD. Recent molecular, genetic and epigenetic evidence indicate that at least 4 miRNAs including the NF-κB-regulated miRNA-9, miRNA-125b, miRNA-146a and miRNA-155 are progressively up-regulated in both AD and age-related macular degeneration (AMD). Our prior investigation has asserted that this quartet of up-regulated miRNAs down-regulate a small brain- and retinal-cell-relevant family of target mRNAs, including that encoding complement factor H, a major negative regulator of the innate-immune and inflammatory response, and synapsin-II a critical neurotransmitter release protein. In this study, we are investigating miRNA expression in AD and age-matched controls by specifically concentrating to find discriminatory miRNA-146a, miRNA-9, miRNA-125b and miRNA-155 patterns with other co-regulated miRNAs from hippocampal and temporal lobe tissue samples. We have developed

feature selection methods to rank their abundances, which highlight the differentially expressed miRNAs. Further, we employ diverse statistical measures to identify the differentially expressed miRNAs with the quartet as our miRNAs of interest. In a related, but different study, we have demonstrated a comprehensive analysis of gene interactions using the weighted gene co-expression network analysis that can identify modules of co-expressed genes that are functionally similar and gauge the conservation of these co-expressed genes as the disease progresses.

2:10 - 2:30 pm

Antiproliferative effects of combination treatment of γ-tocotrienol with PPARγ agonists and antagonists can be mediated through PPARγ-independent mechanisms in +SA breast cancer cells

Abhita Malaviya, Dr. Gus Kousoulas and Dr. Paul W. Sylvester
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γ-Tocotrienol is a member of the vitamin E family that displays potent antiproliferative effects against breast cancer cells. Peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor family that upon activation by endogenous binds to specific PPAR response elements (PPRE) and acts as a transcriptional regulator. Studies have shown that treatment with the combined treatment of γ-tocotrienol with PPARγ agonists, upregulated the expression of PPARγ while combination treatment of γ-tocotrienol with PPARγ antagonists, downregulated the expression of PPARγ, thus making a potent anticancer combination against human breast cancer cells working via PPARγ. However, some studies also suggest that PPARγ ligands can also work independent of PPARγ; therefore, this present study was conducted to understand whether combination treatment of γ-tocotrienol with PPARγ ligands can work independent of PPARγ. For this purpose, combination treatment studies of γ-tocotrienol with PPARγ agonists and antagonists were performed on mouse +SA mammary tumor cells that are PPARγ negative. Treatment alone with high doses of PPARγ agonist or high doses of PPARγ antagonists had no affect on the growth of breast cancer cells. However, combined treatment with subeffective doses of γ-tocotrienol with PPARγ agonists was found to increased cell growth, whereas, the combined treatment with subeffective doses of γ-tocotrienol with PPARγ antagonists was found to significantly inhibited growth of +SA cells. In addition, qRT-PCR results showed that combined treatment of γ-tocotrienol with PPARγ antagonists decreased expression of cyclooxygenase-2 (COX-2) along with upregulating expression of prostaglandin D2 receptor (PGD2) and PGJ2 that was followed by cell cycle arrest in +SA breast cancer cells. These outcomes strongly suggest that combination treatment of γ-tocotrienol and PPARγ ligands show potent anticancer effects independent of PPARγ.

2:30 - 2:45 pm

Metagenome Analysis

Mihir Karnik
Louisiana Tech University

Metagenomic studies allow microbial ecologists to determine community composition in an environment overflowing with nonculturable microorganisms. Most environments will contain dominant and non-dominant populations. Defining the less abundant members of a complex community is not a trivial undertaking - the more complex an environment, the more sequencing will be necessary to define that community.

The process of defining the less abundant members of a complex community is done by using a file containing all sequences from a mock community with ambiguous bases present in them. This is followed by removing the sequences containing ambiguous bases to find sequences which mapped to reference sequences for different organisms and output these sequences. The next step is to identify the unique sequences i.e. to remove all the sequences that do not uniquely map to a

particular reference sequence, and count them before outputting them in a separate file. Then we find the number of times that each sequence that uniquely mapped to a particular reference sequence is present in the entire read file and consolidate all the outputs and creating a file which will tell which unique sequence maps to what reference sequence and the number of times it is repeated.

The results that have been obtained using the programs give us an idea as to the quantified amount of different organisms present in the sequence under consideration which help us to find out the least abundant organism in a particular community along with also how it compare to the most abundant organisms.

The way of testing the results is to use software that have already been tested and found to have been accurately predicting the concentration of different organisms in a community.

Future work would entail working on real-world communities along with developing algorithms that would enable us to carry out our methodology in a faster and more accurate manner.

2:45 - 3:00 pm

Effect of Various Transformations on the Responsive Distribution and on Gene Selection on Gene Expression Data for Gastric Cancer

Kankana Shukla, Dr. Prerna Dua and Dr. Hilary W. Thompson
Louisiana Tech University and LSUHSC-New Orleans

Gastric cancer has been linked to various factors but in spite of intensive research, its fundamental cause remains unknown. Most patients of gastric cancer are asymptomatic, making the diagnosis of the disease challenging. Gene expression analysis techniques have clinically proved to improve diagnosis and prognosis of diseases. Thus gene expression analysis has a high potential to considerably contribute in the early detection of the stomach cancer. One of the common challenges in analyzing DNA microarray data is the curse of dimensionality. Additionally, the presence of noise and complex biological interactions in a gene network further complicates the analysis of data expression data. Thus, for accurate classification of the disease, discovery of a subset of genes that contrast samples is crucial for understanding the underlying carcinogenic process.

In this work, we analyzed a previously never used gene expression data obtained from 569 adults (361 African Americans and 208 Caucasians) undergoing gastric biopsies for genomic DNA extraction. We undertook linear, logarithmic and cubic-spline preprocessing techniques for data transformation and compared their performances by constructing the corresponding classifier models. We also performed successive feature selection after transformation by the well-known feature selection techniques. We subsequently performed the supervised learning approach proving that the subsets of genes after transformation and feature selection produce better classifier models than the raw data model thus helping in the better classification for gastric cancer. We also came across seven genes that came up in our analysis that are proved to be responsible for gastric cancer in previous researches, validating our computational approaches. Our future work consists of performing pathway analysis on this data and obtain the biological validation.

3:00 - 3:15 pm BREAK

3:30 - 3:50 pm

Dimerization of HERs in breast cancer: dynamics, inhibition and molecular mechanism

Seetharama Satyanarayanajois, S. Banappagari, S. Kanthala, Y.U. Liu and G.M. Vicente
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HER2, a member of EGFR proteins, is overexpressed in approximately 30% of breast cancers. HER2 is known to form heterodimers with other EGFR proteins such as EGFR and HER3, and is a major therapeutic target in breast cancer treatment. Apart from EGFR-HER2, HER2-HER3 is the preferred heterodimer in HER2 overexpressed breast cancer. Extracellular domains II and IV of EGFRs are known to participate in dimerization. Among these domains, domain IV is reported to be clinically relevant. To understand the flexibility of domain IV of EGFRs and their importance in stabilizing the dimerization, we have carried out nanosecond MD simulations using NAMD. We have also designed a number of peptidomimetics to target domain IV of HER2 protein to inhibit HER2-mediated signaling. One of such peptidomimetics, compound 5, exhibited antiproliferative activity with IC50 values in the nanomolar range against HER2 overexpressing breast cancer cell lines SKBR-3 and BT-474. To further investigate the structure-activity relationship of peptidomimetics analogs of 5 were designed. Among various analogs of compound 5, 9 and 18 exhibited antiproliferative activity against breast cancer cell lines in nanomolar range concentration. PathHunter and proximity ligation assay results indicated the inhibition of HER2 heterodimerization by compound 9. Furthermore, in vivo studies in xenograft model of breast cancer suggested that compounds 5 and 9 delayed the breast tumor growth. Compound 5 was conjugated with BODIPY fluorescent probe to evaluate the binding and internalization of 5. These results suggest that small peptidomimetic molecules can inhibit protein-protein interactions of EGFRs, which can be therapeutically useful for controlling breast cancer. Future studies will be focused on identification of binding region of these compounds on domain IV of HER2 and computational studies of heterodimer to understand the molecular mechanism of cell signaling as well as stability of peptidomimetics

3:50 - 4:10 pm

Modeling Protein-Substrate Interactions Using Computational Approaches

Shuju Bai
Southern University and A&M College

Our research has two specific aims. 1) Model protein-Substrate interactions in 8R-lipoxygenase; 2) Test the general applicability of this model to lipoxygenase family. In addition, we apply new computational technology into molecular dynamics. We have finished molecular dynamics simulation of arachidonic acid:8R-lipoxgenase complex to confirm/verify the model we developed using ICM. The results suggest a model which could possibly explain the interactions between 8R-LOX and arachidonic acid. We are in the process of simulating the interactions of 5-LOX and arachidonic acid. Replica Exchange Statistical Temperature Molecular Dynamics (RESTMD) has been finished and performed on LONI clusters. We also implemented RESTMD on a new computational platform, Hadoop, using MapReduce framework. Performance test of our implementation shows scalability and stability on traditional HPC clusters. Future goals include 1) Finish MD simulations of the LOX family members and generate a model. 2) Incorporate MPI features into MapReduce-based MD's to speed up simulation.

4:10 - 4:25 pm

Relationship between retinoic acid and Hoxa1 on the differentiation of mouse ES cells into neuroectodermal versus endodermal lineages

Xiaoping Yi
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Embryonic stem cells (ES cells) are pluripotent cells derived from the inner cell mass at blastocyst stage and can be induced to differentiate into all three primary embryonic germ layers (endoderm, mesoderm, and ectoderm). For instance, the directed differentiation of ES cells along a neuronal pathway can be achieved by treatment ES cells with retinoic acid (RA). Although the effects of RA on cells and tissues are known to occur through the activation of retinoic acid receptors (RAR◎, -◎, and -◎◎and their isoforms), the events occurring downstream of RA signaling that direct the differentiation of ES cells into different cell lineages are poorly understood. In the present work, we have examined the effect of RAR-◎◎ activation on ES cell differentiation and found that this RAR isoform is involved in the activation of the AP1/cJun pathway and on the induction of visceral endodermal differentiation. RAR-◎◎ was also shown to repress Hoxa1 mRNA expression as well as neuronal differentiation. These results suggest that manipulation of RAR-◎◎ activation may constitute a novel strategy for the directed differentiation of ES cells into endodermal versus neuroectodermal lineages.

4:25 - 4:40 pm

Synthesis of Petides designed from CD 2 strand to modulate protein-protein interactions in Rheumatoid Arthritis

Ameya Gokhale, Dr. Seetharama Jois and Dr. Gus Kousoulas
University of Louisiana at Monroe and LSU-BR

The protein-protein interaction between CD2 on T cells and CD58 on antigen presenting cells (APC) is important in the early stage of immune response. Co-stimulatory/adhesion molecules play important roles in the tight adhesion of T cells to APC. Protein-protein interaction between CD2 and CD58 helps enhance T cell-APC adhesion and thus promotes T-cell activation. Blockade of the CD2-CD58 interaction leads to immunosuppression both in model systems and in humans, indicating the importance of CD2-CD58 interaction for the cellular immune response. The primary aim of our research is to design and synthesize peptides that are derived from the surface epitopes of CD2 protein which will bind to CD58 and block the adhesion interaction of T cells and target cells and, hence modulating the immune response. The two specific aims in the summer research projects were, 1) To synthesize designed peptides using solid phase peptide synthesis, and , 2) To generate recombinant human CD58 protein in E.coli and purification of protein. The peptides were synthesized using solid phase peptide synthesis (SPPS) on an automatic peptide synthesizer as well as microwave assisted manual synthesis using Fmoc strategy at LSU protein facility. Cyclic peptides were synthesized on the resin using side chain linked resins using an orthogonal strategy. The peptides were characterized by electro-spray mass spectrometry (ESI-MS) and analytical RP-HPLC. Human CD58 adhesion domain (hCD58AD) fusion protein was expressed in Escherichia coli. Cloning of full length hCD58AD cDNA using the amino acid sequence of CD58 in the PDB, code 1CI5A, Chain A, Glycan-Free mutant adhesion domain of human CD58, and reverse translating the gene using high codon usage for E coli was performed. The cDNA was transfected in E. coli and stable expression of CD58 protein was achieved. The protein was isolated and purified by gel-filtration chromatography.

Notes

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LBRN Upcoming Events

February 15, 2013	LBRN Summer Research Program Application Deadline
February 11, 2013	LBRN Annual Progress Report and PHS2590 Deadline
TBD	Resume LBRN Work-in-Progress via Access Grid
February 1, 2013	Southeast INBRE Region Program of Distinction Deadline
May 27 - August 2, 2013	LBRN Summer Research Program
August 2, 2013	Summer Undergraduate Research Forum (SURF)
October 2013 - TBD	External Advisory Committee Meeting
January 2014 - TBD	LBRN 11th Annual Meeting

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