TYPE III IFN (IFN-LAMBDA) ANTIVIRAL ACTIVITY AGAINIST HCV IN AN IFN-ALPHA RESISTANT CELL LINE INVOLVES miR-122 TARGETS


*Pathology and Laboratory Medicine, ″Department of Medicine, Gastroenterology and Hepatology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA-70112.

Background: Recent studies indicate that polymorphism of Interferon lambda (IFN-λ) gene is strongly associated with the natural clearance of HCV infection and interferon/ribavirin treatment response in chronic HCV patients. A recent publication from our laboratory indicates that HCV replication in the persistent cell culture remain resistant to IFN-alpha whereas IFN-lambda induces viral clearance. The mechanism by which the lambda induces HCV clearance is unclear.

Aim: To study the unique antiviral mechanism by which IFN-λ clear HCV replication in an interferon alpha resistant HCV sub-genomic replicon cell culture.

Methods: Interferon alpha sensitive (S3-GFP) and resistant (R4-GFP) cells were treated with IFN-λ1 (1 to 100 ng/ml) and IFN-α (10-100IU/ml) for 72 hours. The antiviral effect of IFN-λ1 was determined by measuring the HCV replication by G-418 resistant cell colony, HCV GFP fusion protein expression by flow cytometry and viral RNA levels were measured by real time RT-PCR. The signaling pathways, interferon stimulated gene (ISG) expression, and miRNA regulated by IFN-λ1 in R4-GFP cells were measured by Western blot analysis, real time RT-PCR and miRNA microarray.

Results: We show that IFN-λ1 inhibits HCV replication in a sub-genomic R4-GFP replicon cell line resistant to IFN-α in a dose dependent manner. IFN-λ1 induced phosphorylation of STAT1, STAT2 and STAT3 as well as activation of the interferon beta (ISRE) promoter in this cell line in a dose dependent manner. IFN-λ1 treatment induced a set of known interferon stimulated genes (PKR, OAS and MxA) in R4-GFP cells. Our results show that only STAT2 inhibition blocked the antiviral effect of IFN-λ1. MiRNA array analysis revealed that a wide range of miRNAs were either induced or inhibited in R4-GFP replicon cell lines after IFN-λ1 treatment. Northern blot analysis indicated that IFN-lambda treatment of R4-GFP cells reduced expression of miR-122. Flow analysis experiment showed that co-treatment with miRNA-122 mimic prevented IFN-lambda mediated suppression of GFP expression in R4-GFP cells. Furthermore, a significant decrease in HCV GFP expression was achieved when miRNA-122 inhibitor added together with IFN-lambda in R4-GFP cells.

Conclusions: These results demonstrate that IFN-λ1 inhibits HCV replication in an IFN-α resistant cell line by a unique antiviral mechanism involving the regulation of miRNA-122. Our results suggest that IFN-λ1 can be effectively used to treat chronic HCV infection in those not responding to IFN-α therapy.
MECHANISMS OF ESTROGEN SUPPRESSION OF HYPERGLUCAGONEMIA IN THE INSULIN-DEFICIENT DIABETIC MOUSE

Allard C*, Tate CR*, Mauvais-Jarvis F*

* Division of Endocrinology and Metabolism, Department of Medicine, Tulane University Health Sciences Center, New Orleans, LA

A large body of evidence implicates hyperglucagonemia as instrumental in the maintenance of unsuppressed hepatic glucose production and therefore hyperglycemia in both type 1 (T1D) and type 2 diabetes (T2D). Thus, suppressing hyperglucagonemia is a major therapeutic strategy for controlling hyperglycemia in diabetes. During chronic insulin deficiency and hyperglycemia, insulin suppression of glucagon secretion is lost. Thus, restoring insulin ability to suppress glucagon secretion would represent a powerful novel therapeutic approach. Estrogen (E2) is known to protect functional β-cell mass in diabetes. Our lab reported that E2 treatment improves human pancreatic islet transplantation in diabetic mice with dramatic improvement in hyperglycemia starting one day after islet transplantation. E2 suppression of hyperglycemia was not due to an increase in insulin secretion but was associated with a pronounced suppression of hyperglucagonemia. We showed that the estrogen receptor α (ERα) is expressed in mouse and human α-cells. Further, our experiments suggest that ERα needs to be activated in α-cells of the host pancreas since the hypoglycemic effect of an ERα-selective agonist is lost when islets from WT mice are transplanted in ERα-null mice (lacking ERα in α-cells). Accordingly, the ERα-agonist can still acutely suppress blood glucose and improve diabetes when ERα-deficient islets are transplanted in WT mice.

To test whether E2 synergizes with insulin to suppress hyperglucagonemia in the recipient insulin-deficient diabetic mouse α-cells, we used male C57Bl/6 mice rendered diabetic with streptozotocin (STZ, 200 mg/kg). These mice were treated with vehicle, E2, insulin (Ins) or Ins+E2. Treatment with Ins+E2 produced a stronger suppression of hyperglycemia compared to Ins alone. However, glucagon levels were not modified between all the groups.

Thus E2 improves insulin sensitivity to suppress hyperglycemia in diabetic mice independently from a decrease in hyperglucagonemia. These results suggest that E2 suppression of hyperglucagonemia in diabetic mice requires the presence of transplanted islets rather than insulin alone. Studies are ongoing to determine the identity of the islet factor(s) that synergize with E2 to suppress hyperglucagonemia.

This work is funded by an American Diabetes Association Grant (7-13-BS-101).
PIOGLITAZONE IS PROTECTIVE OF ERECTILE FUNCTION IN A RAT MODEL OF POST-PROSTATECTOMY ERECTILE DYSFUNCTION

Authors: Aliperti LA1, Lasker GF2, Hagan SS1, Hellstrom JA1, Walter KA2, Kadowitz PJ2, Trost LW3, Sikka S1 and Hellstrom WJG1

Addresses: 1Department of Urology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112-2699, USA
2Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA
3Department of Urology, Mayo Clinic, Rochester, MN

Introduction: Erectile dysfunction (ED) is a common comorbidity following radical prostatectomy. In addition to existing therapies, several investigations are ongoing into novel pharmacotherapeutic treatment options. Pioglitazone (PIO) is a thiazolinedione (TZD) commonly utilized in the treatment of diabetes mellitus (DM). Given its known vasculoprotective and antifibrotic properties, we sought to evaluate the efficacy of PIO on ED in a rat nerve-crush model of ED.

Materials and Methods: Fifteen Sprague-Dawley rats were stratified into three groups: 1-sham, 2-nerve crush (NC), 3-PIO treatment. Sham rats underwent an abdominal incision with no further surgery. Groups two and three underwent bilateral cavernosal nerve crush. All rats subsequently underwent oral gavage for 14 days (sham and NC with phosphate buffered saline (PBS), PIO treatment with PIO 0.65 mg/kg). Following a one-day washout period, all rats underwent bilateral cavernosal electrical stimulation at 2.5, 5.0, and 7.5V. Intracavernosal pressure to mean-arterial pressure (ICP/MAP) was assessed. Statistical comparisons were performed using student's t-test, with p<0.05 considered significant.

Results: Significant decreases in ICP/MAP were noted with NC rats compared to sham animals (sham vs crush: 2.5V - 0.65±0.09 vs 0.25±0.05; 5V - 0.71±0.08 vs 0.35±0.07; 7.5V 0.77±0.06 vs 0.42±0.05, p-value. PIO-treated animals had significantly
increased ICP/MAP values compared to NC controls (PIO vs NC: 2.5V - 0.47±0.07 vs 0.25±0.05 p=0.0422; 5V - 0.57±0.05 vs 0.35±0.07 p=0.0343; 7.5V - 0.62±0.05 vs 0.42±0.05, p=0.0229.)

**Conclusion:** PIO administration results in improved erectile function in a rat model of post-prostatectomy ED. Further evaluation is required to identify potential mechanisms of action and assess clinical utility.

**Grant:** Sexual Medicine Society of North America Medical Student Grant.
Early Prediction of Impending Recurrent Laryngeal Nerve Injury during Neck Surgery by Continuous Intraoperative Vagus Nerve Monitoring

Alsaleh Nuha MD, FRCSC, MSc; Mohammad A. Murcy, MD; Salah Eldin H. Mohamed, MD; Paul L. Friedlander, MD, FACS; Rizwan Aslam, DO, MS; FACS; Emad Kandil, MD, FACS

Department of Surgery, Tulane University School of Medicine, New Orleans, LA 70112, USA

Conflict of Interest: Authors have no conflict of interest.

Background: Continuous intraoperative nerve monitoring using automatic periodic stimulation (APS) of the vagus nerve (VN) can recognize early change in function of the recurrent laryngeal nerve (RLN). The purpose of the current study is to examine our initial experience using this technology.

Methods: A retrospective review of the database of 205 consecutive patients who underwent thyroid surgery by a single surgeon using APS at a single North American institution. Stretch injury was established by a warning threshold alarm 50% reduction in amplitude and/or 10% increase in latency. Preoperative and postoperative laryngoscopy was performed for all patients.

Results: A total of 274 RLNs were at risk. There was no change in the signals of 245 (89.5%) nerves. For the VN, the initial and final stimulation signals (± SD) of amplitude were 728.77 ± 408.24 mV, 678.4 ± 495.95 mV with latency of 4.92 ± 1.81 ms, 5.02 ± 4.49 ms, initial and final signals, respectively. For the RLN, the signals (± SD) of amplitude were 998.75 ± 443.66 mV, 969.02 ± 463.03 mV; latency 1.9 ± 0.7 ms, 1.9 ± 0.7 ms, initial and final signals, respectively. APS alarm detected impending stretch nerve injury in 22 (8%) cases by 63.9 (± 13.4) % mean decrease in amplitude and by 27.3% increase in latency in one case. A total loss of signal (LOS) has been detected in 7 (2.5%) cases. The early change of management by releasing the causative retraction for an average of 2 (± 0.7) minutes successfully preserved the nerves in all cases with impending injury; however, there was no improvement in the LOS cases. Other than the cases with LOS, postoperative laryngoscopy showed normal vocal cord function in all cases.

Conclusions: APS technology is safe, feasible and can help in early recognition of intra-operative RLN stretch. Future studies are warranted to further examine the benefits of this technology.

Keywords: Automatic Periodic Stimulation, APS, Continuous Intraoperative Nerve Monitoring, Recurrent Laryngeal Nerve Injury
The neighborhood context has been shown to be an important factor in many health outcomes including HIV and sexually transmitted infection (STI) risk behaviors. Although Latino migrant men represent a high-risk group for engaging in HIV/STI risk behaviors, the neighborhood environment has not been well studied among this population. The goal of this study is to determine if neighborhood composition, as measured by concentrated disadvantage and immigration concentration, is associated with HIV/STI risk behaviors among Latino migrant men living in New Orleans, and if social support, social capital, and time in New Orleans act as effect modifiers or mediators of this relationship. A convenience sample of 251 Latino migrant men was recruited. Participants provided information about their HIV/STI risk behaviors including sexual behavior, drug use, and binge drinking. Neighborhood measures of concentrated disadvantage and immigration concentration were calculated using a factor analysis of census tract data from the American Community Survey 5-year estimates. Buffers of four radii ranging from 0.25 miles to 1.5 miles were drawn around participants’ addresses as a representation of each participant’s neighborhood exposure. A weighted average of the census tract-level concentrated disadvantage and immigration concentration within the buffers was calculated. Logistic regression with robust standard errors was used for statistical analysis. Overall, all participants were living in areas with high immigration concentration; however immigration concentration was not associated with any HIV/STI risk behavior. After adjusting for relevant confounders, drug use and sex with a female sex worker were positively associated with concentrated disadvantage at all four buffer radii. The odds ratios (OR) and 95% confidence intervals (CI) for the relationship between concentrated disadvantage and sex with a sex worker ranged from OR 2.05 (95% CI 1.12, 3.75) at the 0.25-mile buffer to OR 3.18 (95% CI 1.22, 8.26) at the 1.5-mile buffer. The effect sizes for the association between drug use and concentrated disadvantage ranged from OR 2.10 (95% CI 1.23, 3.60) at the 0.25-mile buffer to OR 4.35 (95% CI 1.52, 12.44) at the 1.5-mile buffer. Time in New Orleans was not found to be an effect modifier. Social support partially mediated the relationship between concentrated disadvantage and drug use at the 1.5-mile buffer only. Living in an area with higher concentrated disadvantage appears to place Latino migrant men at an elevated risk of engaging in HIV/STI risk behaviors. These findings were robust to the definition of neighborhood. This study adds to the small existing literature on the neighborhood social environment and HIV/STI risk behaviors among Latino migrants.

This research was supported by a National Research Service Award from the NIH/National Institute on Drug Abuse (F30 DA033729), the Interdisciplinary Research Training Institute on Hispanic Drug Abuse (R25 DA026401), and several R21 awards (R21 DA026806 and R21 DA030269, PI: Kissinger)
EARLY PREDICTION OF IMPENDING RECURRENT LARYNGEAL NERVE INJURY DURING NECK SURGERY BY CONTINUOUS INTRAOPERATIVE VAGUS NERVE MONITORING

Alshehri MH, Alsaleh NA, Murcy MA, Mohamed SH, Friedlander PL, Aslam R, Kandil E,

Department of Surgery, Tulane University School of Medicine, New Orleans, LA 70112, USA

Conflict of Interest: Authors have no conflict of interest.

Background: Continuous intraoperative nerve monitoring using automatic periodic stimulation (APS) of the vagus nerve (VN) can recognize early change in function of the recurrent laryngeal nerve (RLN). The purpose of the current study is to examine our initial experience using this technology.

Methods: A retrospective review of the database of 205 consecutive patients who underwent thyroid surgery by a single surgeon using APS at a single North American institution. Stretch injury was established by a warning threshold alarm 50% reduction in amplitude and/or 10% increase in latency. Preoperative and postoperative laryngoscopy was performed for all patients.

Results: A total of 274 RLNs were at risk. There was no change in the signals of 245 (89.5%) nerves. For the VN, the initial and final stimulation signals (± SD) of amplitude were 728.77 ± 408.24 mV, 678.4 ± 495.95 mV with latency of 4.92 ± 1.81 ms, 5.02 ± 4.49 ms, initial and final signals, respectively. For the RLN, the signals (± SD) of amplitude were 998.75 ± 443.66 mV, 969.02 ± 463.03 mV; latency 1.9 ± 0.7 ms, 1.9 ± 0.7 ms, initial and final signals, respectively. APS alarm detected impending stretch nerve injury in 22 (8%) cases by 63.9 (± 13.4) % mean decrease in amplitude and by 27.3% increase in latency in one case. A total loss of signal (LOS) has been detected in 7 (2.5%) cases. The early change of management by releasing the causative retraction for an average of 2 (± 0.7) minutes successfully preserved the nerves in all cases with impending injury; however, there was no improvement in the LOS cases. Other than the cases with LOS, postoperative laryngoscopy showed normal vocal cord function in all cases.

Conclusions: APS technology is safe, feasible and can help in early recognition of intraoperative RLN stretch. Future studies are warranted to further examine the benefits of this technology.

Keywords: Automatic Periodic Stimulation, APS, Continuous Intraoperative Nerve Monitoring, Recurrent Laryngeal Nerve Injury
SELF-REPORT OF TROUBLE SLEEPING BY RACE/ETHNICITY IN PREGNANT WOMEN AND WOMEN OF CHILD-BEARING AGE

Amyx M*, Xiong X*, Xie Y*, Buekens P*

*Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA

Racial/ethnic differences have been reported in both prevalence and reporting of sleep disorders, but little research has been done examining sleep disorders by race/ethnicity in pregnant women and women of child-bearing age. The purpose of this secondary analysis of data from the National Health and Nutrition Examination Survey (NHANES) from 2005-2010 was to examine report of trouble sleeping to a physician and inadequate sleep (≤5 hours) by race/ethnicity in pregnant (N=432) and non-pregnant women (N=3175) of childbearing age (15-44 years old). The proportion who reported trouble sleeping, inadequate sleep time, and both trouble sleeping and inadequate sleep was estimated by race/ethnicity, stratified by pregnancy status. The differences in the proportions by race/ethnicity were tested using the Rao-Scott χ² statistic. In both pregnant and non-pregnant women, non-Hispanic white women (17.6% and 27.2% respectively) were more likely to have reported trouble sleeping than Mexican-American (9.2% and 10.0%) or non-Hispanic black women (11.4% and 19.6%), though the difference was only significant in non-pregnant women (p<0.01). In contrast, in both groups, non-Hispanic black women (19.7% pregnant and 22.7% non-pregnant) were significantly more likely to report inadequate sleep than non-Hispanic white (3.4% and 11.0%) and Mexican-American women (7.9% and 10.6%). Among women with inadequate sleep, non-Hispanic white women (37.9% pregnant and 51.8% non-pregnant) were most likely to report trouble sleeping, as compared to non-Hispanic blacks (16.1% and 27.8%) and Mexican-Americans (26.4% and 22.9%, p<0.01). In conclusion, non-Hispanic white women were more likely to report trouble sleeping to a physician, while non-Hispanic black women were more likely to report inadequate amounts of sleep. Further, non-Hispanic white women were more likely to have reported trouble sleeping to a physician than minority women getting the same amount of sleep.

This work was supported by an NIH training grant (NIH-5T32HD057780-04).
Promoter DNA Methylation of the CLOCK Gene is associated with Subclinical Atherosclerosis: A Monozygotic Twin Study

An Q*, Goldberg J***, Bremner JD****, Vaccarino V***** Zhao J*

*Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA;
**Seattle Epidemiologic Research & Information Center, Veterans Affairs Office of Research & Development, Seattle, WA;
***Department of Epidemiology, University of Washington, Seattle, WA;
****Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA
*****Department of Epidemiology, Emory University School of Public Health, Atlanta, GA

Background: Epigenetic factor plays a critical role in obesity and its related metabolic disorders such as atherosclerosis. Dysregulation of circadian rhythm has been implicated in both obesity and atherosclerosis. The Circadian Locomotors Output Cycles Kaput (CLOCK) gene is a candidate gene for obesity, and genetic polymorphisms in CLOCK gene has been associated with body weight regulation and body composition measurements. However, the potential role of DNA methylation variation in CLOCK gene in susceptibility to obesity and atherosclerosis has not been investigated so far. The goal of this study is therefore to examine whether DNA methylation variation in the promoter region of the CLOCK gene is associated with subclinical atherosclerosis as measured by brachial artery flow-mediated dilation (FMD), independent of obesity. Moreover, we test whether genetic factors may potentially confound the association of CLOCK gene methylation with subclinical atherosclerosis.

Methods: This analysis included 84 apparently healthy middle-aged male-male monozygotic (MZ) twin pairs from the Emory Twins Heart Study (ETHS). Subclinical atherosclerosis was assessed by brachial artery FMD by bi-mode ultrasound. Methylation levels at 30 CpG sites in the promoter region of CLOCK gene were quantified from peripheral blood leukocytes DNA by bisulfite pyro-sequencing. To examine the association of methylation variation with subclinical atherosclerosis, we conducted pair-wise analysis using intra-pair differences. This was done by regressing the intra-pair difference in FMD on intra-pair difference in DNA methylation level (independent variable) at each CpG site, adjusting for intra-pair differences in body mass index (BMI), high-density lipoprotein (HDL), low-density lipoprotein (LDL), systolic blood pressure (SBP), smoking pack-year. Here intra-pair difference was defined as the actual difference in DNA methylation level at each CpG site or FMD between two members of a twin pair. Multiple testing was adjusted by false discover rate-adjusted q-value, and q-value <0.05 is considered to be statistical significance. All statistical analyses were performed by SAS 9.3.
Results: The mean age of the twin participants was 55±2.8 years old. The mean methylation level of all 30 CpG sites was 1.4290% ± 0.5453% (ranging from 0.3640% to 2.9383%). The mean FMD was 0.0528% (ranging from 0.0016% to 0.1752%). Methylation levels at 10 CpG sites were significantly correlated with each other ($r^2=0.0618 - 0.1478$, p<0.05). Intra-pair difference in FMD was correlated with intra-pair difference in DNA methylation level at one CpG site ($r^2=0.1577$, p=0.0002). Results show that increased intra-pair difference in methylation level at one CpG site was significantly associated with decreased intra-pair difference in FMD (β= -0.0089, [95%CI -0.0130, -0.0047], q-value 0.0024). Specially, per 10% increase in methylation was associated with a 0.089% decrease in FMD.

Conclusion: This study was the first time to demonstrate that methylation variation in the CLOCK gene promoter is associated with subclinical atherosclerosis, independent of genetic and obesity as well as other coronary risk factors. Because monozygotic twins match exactly on genetic background, the use of MZ twin pairs for epigenetic analysis totally eliminates the confounding by genes. Moreover, identical twins in general raised in same environment. This provides further controlled the confounding by early life experience, which has a long-lasting impact on the epigenetic plasticity of human genome.

This study was supported by American Heart Association grant 0730100N and National Institutes of Health grants R21-HL-092363, K01-AG-034259, K24-HL-077506, R01-HL-68630, and R01-AG-026255.
ABSTRACT

Breast cancer is most prevalent cancer women and almost 70% of the cases are estrogen receptor α (ERα) positive. ERα activity is regulated by phosphorylation at various sites; especially serines 118 and 167 have been major focus for many years. Retrospective studies have shown that elevated ERα phosphorylation at serines 118 (S118) and 167 (S167) is associated with favorable outcome for tamoxifen adjuvant therapy and may serve as surrogate markers for a functional ERα signaling in breast cancer. Functional estrogen receptor is the best predictor for the response to anti-estrogen agent like tamoxifen. Since ERα signaling is critical for ERα positive breast cancer cell growth, loss of phosphorylation at S118 and/or S167 could disrupt ERα signaling, which could have an impact on cancer cell proliferation. In the present study, ERα positive MCF-7 breast cancer cells were stably transfected with an ERα specific short hairpin RNA (shRNA) that reduced endogenous ERα subsequently this cell line was stably transfected with wild-type ERα (ERα-AB cells), or ERα containing serine (S) to alanine (A) mutation at S118 or S167 (ERα S118A cells and ERα S167A cells, respectively). All three ERα-AB, ERα S118A, ERα S167A stable cell lines expressed approximately equivalent ERα compared with parental MCF-7 cells. These stable cell lines were evaluated for tumor growth in nude mice. ERα S118A MCF-7 cells displayed a similar tumor growth pattern comparable with ERα-AB cells. ERα S167A MCF-7 cells exhibited a significant decrease in tumor growth compared to ERα-AB MCF-7 cells. Attenuated phosphorylation of ERα at S167 but not S118 significantly decreased the tumor growth of MCF-7 breast cancer cells. At present the mechanism for the reduced tumor growth in ERα S167A cells is not clear. Studies examining the ERα activity, proliferation index, apoptosis, angiogenesis, and EMT markers are under progress.
THE RELATIONSHIP BETWEEN SOCIAL SUPPORT AND PHYSICAL ACTIVITY IN A LOW-INCOME AFRICAN AMERICAN INNER-CITY NEIGHBORHOOD

Andersen L*, Gustat J**, Becker AB***

* Department of Global Community Health and Behavioral Sciences, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA
** Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA
*** Mary Ann and J. Milburn Smith Child Health Research Program, Ann and Robert H. Lurie Children’s Hospital of Chicago Research Center, Chicago, IL

Physical inactivity is related to many diseases yet many Americans are not meeting the physical activity (PA) recommendations. Social support may be one mechanism that increases PA in an African American (AA) adult population. Several areas of social support exist, including receiving help around the house, ability to borrow a car (general support) as well as encouragement by friends/family to be physically active (specific support). The purpose of this study was to examine the relationship between two dimensions of support and self-reported leisure-time PA.

A total of 497 household interviews assessing PA and the community and social environment were conducted with adults in three, low-income, primarily AA urban neighborhoods in New Orleans, Louisiana. Logistic regression models were developed to assess demographic characteristics, knowledge of PA benefits, general support, and support specific for PA. Factor analysis was used to create scales assessing support.

Just over half the sample met the recommendations for PA. Females were less likely to meet PA recommendations compared to males (OR: 0.47, CI: 0.38-0.70) and an inverse relationship existed between age and PA (OR: 0.97, CI:0.95-0.98). Social support specific for PA (OR: 1.10, CI: 1.02-1.14), and being in a romantic relationship (OR: 1.53, CI: 1.03-2.28) were significantly related to PA after controlling for gender and age.

These findings suggest the social environment is an important component of encouraging AA adults to be physically active. PA interventions should consider fostering social networks specific for PA to increase the number of AA adults that are physically active.

This study was part of the core research project of the Prevention Research Center at Tulane University School of Public Health and Tropical Medicine and was funded by the Centers for Disease Control and Prevention Cooperative Agreement Number #1-U48-DP-000047. The findings and conclusions in this abstract are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Estrogen receptor-mediated enhancement of vascular function and potential sex-specific implications

Authors: H.R. Ansari and M.H. Hamblin.

Abstract:
Cardiovascular disease is the leading cause of mortality in the United States. Estrogen receptor (ER) signaling has been previously shown to play a role in vasculoprotection. However, the notion that potential sex differences in estrogen receptor-mediated vascular function exist is underappreciated. Our laboratory examined the effect of ER-alpha activation on phenylephrine (Phe; 10-6 M)-induced precontracted male and female mouse aortas. We found the ER-alpha agonist, PPT induced aortic relaxation in both male and female mouse aortic rings at concentrations of 100 μM (62% ± 4 for males vs. 86.6% ± 5 for females) and 1000 μM (70% ± 6 for males vs. 99.9% ± 6 for females), respectively. The ER-alpha antagonist, MPP obviated PPT-induced aortic relaxation (100 μM) in these endothelial-intact mouse aortas. However, in endothelium-denuded mouse aortas, PPT-induced relaxation was attenuated in both male and female vessels, and there were no sex differences. Next, attenuated PPT-induced aortic relaxation was observed when treated with the ERK1/2 inhibitor, PD98059 or +K-ATP channel blocker, glibenclamide in endothelial-intact male and female aortas. In summary, our pilot data show potential sex differences in estrogen receptor-mediated mechanisms for aortic relaxation and vascular function.
MISTAKEN IDENTITY: MISDIAGNOSIS BASED ON AUTO-ANTIBODIES IN IVIG

Anthony K, El-Dahr J

Allergy-Immunology, Tulane University, New Orleans, LA

IVIG is used to treat a variety of immune mediated conditions. The immunology community is aware that auto-antibodies prevalent in the healthy general population may be contained in IVIG, resulting in false positive serum tests in individuals receiving gammaglobulin. This may be less well known by other specialties. We present a case suggesting increased awareness is needed. An 11 y.o. female presented with a generalized seizure and progressive psychiatric symptoms. CBC, CMP, and ESR were normal. CSF studies included bacterial culture, viral studies, and NMDA receptor antibody. She was given high dose IVIG due to her severe neuropsychiatric symptoms. With continued worsening, additional studies were sent including anti-thyroid antibodies and thyroid function tests. Imaging included CT and MRI/MRA of the brain and CT abd/pelvis. CSF had a mild leukocytosis; viral studies and bacterial cultures were negative. Imaging was unremarkable. After treatment with IVIG, anti-thyroid antibodies were positive with normal thyroid function and she was diagnosed with Hashimoto’s Encephalopathy. Consulting neurologists from multiple centers were queried and all felt that the antibodies could not have come from IVIG. Subsequently however, NMDA receptor antibody from the initial pre-treatment CSF studies returned positive. Subsequent anti-thyroid antibodies were negative. Positive anti-thyroid antibodies in this patient were likely due to IVIG. She was ultimately diagnosed with NMDA Receptor Encephalopathy. Lack of awareness of the transfer of auto-antibodies via IVIG can lead to misinterpretation of lab studies. This case suggests increased awareness is needed among all sub-specialists treating patients with IVIG.
ACUTE AND SUBCHRONIC EFFECT OF OLANZAPINE ON THE SYNAPTIC TRANSMISSION OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS

Anwar IJ*, Miyata K**, Zsombok A***

*Neuroscience Program, Tulane University, New Orleans, Louisiana **Department of Physiology, Tulane University, New Orleans, Louisiana.

Olanzapine, an atypical antipsychotic, alleviates symptoms of schizophrenia while producing fewer side effects compared to first generation antipsychotics. However, chronic usage remains problematic due to the propensity of olanzapine to induce weight gain and metabolic disturbances. Moreover, the cellular mechanisms underlying the metabolic side effects are poorly understood. The central nervous system (CNS) exerts both hormonal and neural control over whole body homeostasis. The dorsal motor nucleus of the vagus (DMV) participates in this regulation through modulation of the parasympathetic outflow to subdiaphragmatic organs. We hypothesized that olanzapine disrupts neurotransmission of the DMV, and thus contributes to the dysregulation of metabolism. We used whole-cell patch-clamp recordings from female C57bl/6 to assess the effect of olanzapine on DMV neurons. First, we investigated the effect of acute olanzapine administration on the activity of DMV neurons. Acute application of 10 µM olanzapine on DMV neurons induced both pre- and post-synaptic effects. Voltage-clamp recordings revealed that, in 5 out of 9 DMV neurons, excitatory inputs to DMV neurons were significantly increased by 50.3±15.6% while the inhibitory inputs were not altered. In addition, in current-clamp mode, olanzapine induced a robust hyperpolarization, in 9 out of 10 neurons, from -48.64 ± 0.68 mV to -57.82 ± 2.47 mV. The hyperpolarization suppressed action potential firing. As a next step, we investigated the subchronic effect of olanzapine on the activity of DMV neurons. Daily subcutaneous injections were made for 20 days (5 mg/kg/day of olanzapine and vehicle). We did not find significant differences in body weight, blood glucose, and insulin or leptin levels. Subchronic administration of olanzapine generated pre-synaptic changes in DMV neurons. In treated animals, additional infusion of 10 µM olanzapine on DMV neurons significantly reduced excitatory neurotransmission by 38.9±3.8% in 10 out of 17 neurons. Our findings indicate that olanzapine directly modulates the neuronal activity in DMV neurons, and could thus contribute to the metabolic disturbances seen in long-term treatments.

This work was supported by Tulane University School of Medicine Research Pilot Project Program.
IMPERMEABLE DUST MITE COVERS IN THE PRIMARY AND TERTIARY PREVENTION OF ALLERGIC DISEASE: A META-ANALYSIS

Arroyave WD*, Rabito FA*, Carlson JC**, Friedman EE*, Stinebaugh SJ*

*Department of Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.
** Department of Pediatrics, Tulane School of Medicine, New Orleans, LA.

Up to 40% of the world's population has been diagnosed with an allergic disease. The most prevalent allergy is to house dust mites. Impermeable mattress covers are often the first treatment in the prevention and decrease of symptoms of allergic disease. Our objective was to perform a meta-analysis evaluating the effectiveness of impermeable mattress covers in the primary prevention of allergic disease and as a single intervention in the tertiary prevention of allergic disease symptoms. MEDLINE, Embase, Web of Science, and CINAHL were systematically searched for relevant publications. Seven primary prevention trials (n = 3,461) and 17 tertiary prevention trials (n = 1,671) met the inclusion criteria and were included in the review. All article reviews and abstractions were performed in duplicate. Our analysis found no significant pooled relative risks for the prevention of allergic disease. The pooled relative risks were 0.97 (95% confidence interval [CI] 0.62-1.51) for house dust mite sensitization, 0.92 (95% CI 0.81-1.05) for wheeze, 0.85 (95% CI 0.70-1.02) for asthma, 1.03 (95% CI 0.90-1.19) for allergic rhinitis, and 1.05 (95% CI 0.84-1.32) for allergic dermatitis. Likewise, no significant pooled standardized mean differences were found in the tertiary prevention of symptoms. The pooled standardized mean differences were -0.03 (95% CI -0.15 to 0.09) for peak flow, -0.06 (95% CI -0.32 to 0.20) for asthma symptom score, and -0.39 (95% CI -0.88 to 0.11) for nasal symptom score. A significant effect was seen in the decrease of house mite dust level in the mattress (-0.79, 95% -0.98 to -0.60). In conclusion, no evidence was found to support the use of impermeable mattress covers in the primary prevention of allergic disease or in the tertiary prevention of allergic disease symptoms.
A NOVEL EX VIVO TISSUE CULTURE ASSAY FOR DETERMINING THE EFFECTS OF ANTI-TUMOR DRUGS ON ANGIOGENESIS

Azimi MS*, Mathur A**, Mondal D**, Murfee WL*

*Department of Biomedical Engineering, Tulane University, New Orleans LA
** Department of Pharmacology, Tulane University, New Orleans, LA

Tumor growth is highly dependent upon angiogenesis and the interplay between the tumor stroma and the microvasculature. A challenge in evaluating anti-tumor therapies is the inability to decouple treatment effects on cancer cells versus the microvascular network. Recently, our laboratory demonstrated that the “rat mesentery culture” model could be used for time-lapse comparison of intact microvascular networks before and after angiogenesis induction. The objective of this study was to demonstrate that this model could be used to evaluate the anti-angiogenic effects for a given drug treatment. Mesenteric windows were harvested from adult male Wistar rats and cultured for 3 days in serum containing medium (MEM + 10% Fetal Bovine Serum). Explants were exposed to increasing concentrations of Nelfinavir (Viracept™) (0 - 4.5 µM) and Curcumin (0 - 4.5 µM). Nelfinavir and Curcumin were selected for the study because Nelfinavir causes endothelial dysfunction and Curcumin, an anti-oxidant and NF-κB inhibitory dietary supplement, is known to have anti-angiogenic effects. Preliminary studies also suggest that the combination of Nelfinavir/Curcumin produce synergistic anti-cancer effects. Labeling tissues with FITC conjugated BSI-lectin on Day 0 and Day 3 identified endothelial cells along all vessels. Results showed significant dose-responsive effects of Nelfinavir/Curcumin combinations in suppressing both vessel segment density and the number of capillary sprouts per vascular area. Our findings establish the rat mesentery culture model as a valuable ex vivo tool for the rapid and reproducible screening of drug effects on angiogenesis in an intact network scenario.

This work was supported by the Tulane Center for Aging and NIH 5-P20GM103629-02 to W.L.M.
NEIGHBORHOOD CRIME, INTIMATE PARTNER VIOLENCE, AND BIRTH OUTCOMES IN PREGNANT WOMEN IN A DISASTER RECOVERY ENVIRONMENT

Barcelona de Mendoza V*, Harville EW*, Giarratano GP**, Savage JS***
*Tulane University School of Public Health and Tropical Medicine, Department of Epidemiology, New Orleans, LA USA
**Louisiana State University Health Sciences Center School of Nursing, New Orleans, LA USA
***Loyola University New Orleans, School of Nursing, New Orleans, LA USA

In post-disaster environments such as New Orleans, neighborhood disruption, crime and intimate partner violence persist. The effects of crime and violence on low birthweight and preterm birth were studied. Women were interviewed at prenatal and community clinics, and those who had singleton births and complete records for at least one exposure, outcome and covariates (n=296) were included in this analysis. Low birthweight and preterm birth were observed in 6.4% and 6.6% of the sample, respectively. Participants who perceived illegal drug use to be a very serious problem in their neighborhood were significantly more likely to have a preterm birth (aOR 5.64, 95% CI 1.2-26.6) than those who did not see it as a serious problem. Women who would not report a crime to police were more likely to have a preterm birth (aOR 9.36, 95% CI 2.2-41.2) and low birthweight baby (aOR 5.98 95%CI 1.5-24.7) than those who would report a crime. The perception that their neighborhood had become less safe in the last year was also associated with increased risk for preterm birth (aOR 3.11 95% CI 1.1-9.0) for participants. Reported domestic violence was not associated with adverse outcomes in this sample. Perceptions of neighborhood crime and safety predicted adverse birth outcomes in this sample and may be considered as areas for intervention for community-based programs.

Funding: NIH, NINR, 5R03NR012052 - 02
PREVALENCE OF HIV-2 AND ART TREATMENT COVERAGE IN NORTHERN SIERRA LEONE

Bond NG*, Goba A**, Levy DC*****, Moses LM***, Sesay SK****, Bangura I****, Gibateh MK****, Khan SH***,*****and Marx PA****,******

*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA; **Tulane University Lassa Fever Project, Kenema, Sierra Leone; ***Tulane University School of Medicine, New Orleans, LA; ****Kabala Government Hospital, Kabala, Sierra Leone; *****Sierra Leone Ministry of Health and Sanitation, Freetown Sierra Leone; ******Tulane University National Primate Research Center, Covington, LA

Introduction/Aim: Acquired immunodeficiency syndrome caused by HIV-1 or HIV-2, affects 35.3 million people worldwide. Nine HIV-2 subtypes originating from sooty mangabeys in West Africa have been described. Subtypes A and B are epidemic while C to I are crossovers that are known in single persons. HIV-2F is an exception among non-epidemic subtypes, being pathogenic and found in two persons, both from Northern Sierra Leone, suggesting transmissibility. Very little data are published concerning the distribution and prevalence of HIV-2 in Northern Sierra Leone despite a new pathogenic HIV-2 emerging from this region. Data on ART treatment coverage is also lacking.

Materials and Methods: Subjects presenting for voluntary HIV test and those referred by healthcare providers were enrolled following informed consent. This represents a targeted, higher risk population than the general population. Commercial HIV-1/2 rapid tests were used in the field. PCR and NGS methods are being used to determine prevalence of newly emerging HIV-2F. A questionnaire was administered to collect demographic information and treatment history in those testing positive.

Results: To date the prevalence of HIV in the targeted sample population is 5.98%. Interestingly, when compared to the last published data on HIV-2 in the region, prevalence has increased by a factor of 32-fold from 0.021 to 0.68%. 77% of HIV positive persons were newly identified cases. Of those previously testing HIV positive, only 41% were currently on treatment compared to 61% ART coverage in HIV positive persons in low and middle-income countries globally.

Conclusions: Our data indicate the prevalence of HIV has increased in Sierra Leone since the civil war. Further data are needed to conclusively show prevalence changes of HIV in Northern Sierra Leone on a population level. The data also show that ART treatment rates in Northern Sierra Leone are significantly lower than the global average highlighting the need for improved case identification and treatment provision in this resource poor setting. Sequencing of HIV positive samples to determine the subtype is in process. This study provides a basis for further population based study of the HIV strains circulating in Northern Sierra Leone.

This work was sponsored by the National Institute of Allergy and Infectious Diseases (NIH-5R01 AI076067).
The physical microenvironment, including cues such as fluid shear stress, tension, and substrate stiffness, have been shown to promote differentiation towards specific phenotypes. These extracellular cues are converted into intracellular signals through the process of mechanotransduction, which includes activation of biochemical pathways and the cytoskeleton. While the exact mechanisms of mechanotransduction are not well understood, it is known that external physical forces are counterbalanced by the cytoskeleton, particularly the actin-myosin motor. Thus, the objective of this study was to determine the effect of intracellular forces on stem cell differentiation using inhibitors of actomyosin contraction and actin polymerization.

Embryonic stem cells (ESCs) were cultured in suspension as embryoid bodies (EBs). EBs were treated during two windows, either early (Day 2-4) or late (Day 4-7), with inhibitors of actomyosin contractions (blebbistatin) or actin polymerization (cytochalasin-D). We found that both inhibitors decreased the expression of pluripotency markers. The inhibitors had little effect during the early treatment window but downregulated gene expression of mesodermal markers during later differentiation. Specifically, markers for the lateral (FLK1) and intermediate (PAX2) mesodermal plates were downregulated at the later time point.

These results indicated that perturbation of intracellular forces via the actin-myosin motor can be used to modulate stem cell differentiation and that a decrease in intracellular force downregulates mesodermal commitment. Further studies of mechanotransduction and the conversion of extracellular to intracellular force may help to elucidate or improve protocols to drive differentiation toward mesodermal or endodermal lineages.
Stromal vascular fraction (SVF) is an emerging and attractive adipose tissue-derived therapy that has shown promise in treating many disorders including inflammatory and immune diseases. SVF contains numerous cell populations with immunomodulatory and regenerative capacity e.g., adipose-derived stromal cells, endothelial precursor cells, T regulatory cells, and monocytes/macrophages. Much evidence has demonstrated attenuation of pathological progression and amelioration of symptoms when introduced into models of autoimmune diseases. For these reasons, SVF is a considerable therapy for Multiple Sclerosis (MS) which is an autoimmune disease characterized by demyelination and widespread inflammation in the central nervous system. For this study, therapeutic administration of SVF in the experimental autoimmune encephalomyelitis (EAE) mouse, a model of MS, provides the advantages of using non-expanded SVF cells as a treatment alternative. Days post induction (DPI) 10 in EAE mice represents a therapeutic time point, i.e., following presentation of symptoms, when one million SVF cells are injected. Preliminary data shows reduction of clinical scoring and improved motor function in SVF-treated EAE mice compared to the vehicle control group. This data suggests that the cells within SVF provide anti-inflammatory effects resulting in modulation of the immune response in EAE. Administration of SVF demonstrates an advantageous therapy that addresses the underlying pathological mechanisms in EAE; thus, proposing SVF as a potential treatment for MS.
MECHANICAL MODULATION OF TRANSPLANTATION SITES FOR STEM CELL-BASED THERAPIES

Broadnax EM*, Sarmiento N*, Van Winter RJ*, Ahsan T*

*Department of Biomedical Engineering, Tulane University, New Orleans, LA

Transplantation of mesenchymal stem cells (MSCs) is thought to be a potential therapy for revascularization of ischemic tissue. Within days of transplantation, however, MSCs are no longer present at the site of delivery. Our primary hypothesis is that this may be due to a sudden change in mechanical microenvironment. For example, after cell culture expansion under static conditions, transplanted cells may be exposed to large deformations in a beating heart or contractile forces of skeletal muscle. We propose that this is of even greater consequence for MSCs from older donors, which would have direct implications for autologous procedures. Thus, the objective of this project is to develop an animal model for regenerative medicine that allows modulation of the mechanical microenvironment in situ. Using the hindlimb ischemia model, we propose to transplant MSCs from donors of various ages into ischemic tissue. These rodents will then be housed in suspension cages similar to ones used in microgravity studies. These cages include an apparatus that will off-load the weight of the rodent hindlimb, resulting in decreased mechanical stresses and strains in the ischemic tissue. Results from studies using this model will increase knowledge that can benefit designs of stem cell therapies for conditions such as myocardial infarction.
GPR30-INDUCED INCREASES IN CYCLIC AMP INVOLVE BOTH GaS AND Gαl/O SUBUNITS

Budish RA, Liu L, Lindsey SH

Department of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana

The most recently discovered estrogen receptor GPR30 is a G protein-coupled receptor that triggers rapid signaling pathways. We previously showed that GPR30 activation increases cyclic AMP (cAMP) in vascular smooth muscle cells and that cAMP signaling is necessary for GPR30-induced vasorelaxation. Therefore, we hypothesized that GPR30 couples to GaS in vascular smooth muscle cells (VSMC). VSMC were treated with inhibitors and activators of G protein signaling before stimulation with the GPR30 agonist G-1 and measurement of cAMP by ELISA. Both G-1 and forskolin increased cAMP compared to DMSO control (P< 0.05, n= 4). NF449, a GaS inhibitor, and pertussis toxin, a Gαl/O inhibitor, attenuated the increase in cAMP from G-1 (P< 0.05, n= 4). Cholera toxin, a GaS activator, did not alter cAMP signaling (P > 0.05, n=4). We conclude that GPR30-induced increases in cAMP may result from activation of both GaS and Gαl/O subunits. The characterization of alpha subunits which couple to GPR30 will help to delineate the signaling mechanisms activated by this membrane-bound estrogen receptor.

Funding: NIH R00HL103974
Recent advances in lung tissue engineering have utilized the extracellular matrix scaffold of lung tissue generated from native lungs by a process known as whole-organ detergent-based decellularization. Bioartificial lungs, which demonstrate the capacity for short-term *in vivo* pulmonary function, have been generated in rodent models by repopulating lung tissue scaffolds with embryonic and fetal stem cells. To evaluate lung tissue engineering in a clinically-relevant large animal model, our laboratory decellularized the lungs of non-human primate rhesus macaques. It is unknown whether the decellularization process affects the spatial properties of large-animal lungs; therefore, a stereological comparison between native and decellularized lungs introduces a novel quantitative method to evaluate the retention of lung architecture after decellularization. Stereological and morphometric analysis of native lungs from a female rhesus macaque of 9.99 years of age yielded estimates for several structural parameters. Values for total lung volume (254 cm$^2$), parenchymal volume fraction (0.9106), alveolar airspace volume fraction (0.5591), mean linear intercept (.01937 µm$^{-1}$), alveolar number (2.11x10$^8$), number-weighted mean alveolar volume (778,051 µm$^3$), and volume-weighted mean alveolar volume (9,872,552 µm$^3$) fell within the range of previously reported values in female rhesus macaques of a similar age. The measurement of alveolar epithelial surface area (98,520 cm$^2$) fell below previously reported values and may reflect differences in the tissue processing method or greater variation among individual animals. An identical stereological description of the decellularized lung from a female macaque aged 14.74 years indicated that the total lung volume of the decellularized lung was 88 cm$^2$ - only 34.65% of the total native lung volume. This markedly decreased volume estimate may reflect changes to the compliance and elastance of the lung following successful removal of cells from the lung after decellularization. With the absence of cells to retain the structure, the combined elastic recoil of the extracellular matrix and inward force applied by tissue embedding and processing may have caused the decellularized lung to compress. Additional parameters of the decellularized lung reflect the reduced total lung volume estimate. In addition, the estimated value for mean linear intercept was 32.83% greater in the decellularized lung than the native lung, indicating a decrease in the space between alveolar septal walls. This change in mean linear intercept is consistent with augmented compression of the alveoli in the decellularized organ. The fractional airspace volume was increased in the decellularized organ by 7.10%, indicating a slight decrease in the volume of alveolar septa relative to airspace following decellularization. In the context of this stereological comparison of decellularized and native macaque lung, future analyses of recellularized tissue can be utilized to quantify the recapitulation of the native organ structure. The ultimate clinical application of decellularization technology in the generation of bioartificial lung requires these initial characterizations in this non-human primate model.
ZEB2 PROMOTES MOTILITY AND METASTASIS IN ER+ BREAST CANCER CELLS


*Department of Medicine, Section of Hematology and Oncology, Tulane University School of Medicine, New Orleans, Louisiana, USA

**Department of Biomedical Engineering, Tulane University, New Orleans, Louisiana, USA

*** Medical Sciences, Indiana University School of Medicine, Bloomington, IN, USA

****Tulane Health Sciences Center and Tulane Cancer Center, New Orleans, Louisiana, USA

While the precise molecular mechanisms underlying metastasis remain unclear, epithelial-to-mesenchymal transition (EMT), the loss of an epithelial cell phenotype and acquisition of a mesenchymal cell phenotype, has been implicated in cancer cell invasion and dissemination. The ZEB family of transcription factors, which includes ZEB1 and ZEB2, has been demonstrated to mediate this transition by downregulating the expression of genes associated with an epithelial phenotype. We sought to investigate the effects of direct ZEB family overexpression on EMT in estrogen receptor-positive (ER+) breast cancer cell systems. We overexpressed ZEB1 or ZEB2 in the epithelial, ER+, luminal A breast cancer cell lines MCF-7 and ZR75. Overexpression of individual ZEB1 and ZEB2 levels were confirmed and localization of the ZEB factors to the nucleus was confirmed by confocal microscopy in both cell lines. ZEB2 overexpressing cells, but not ZEB1 overexpressing cells, showed increased migration and invasion in vitro compared to the vector control in both MCF-7 and ZR75 cell lines, suggesting differential function of the two ZEB family members. Additionally, MCF-7-ZEB2 xenografts exhibited increased lung metastasis compared to MCF-7-vector cells. To elucidate the effects of ZEB on our ER+ cell line we performed next generation deep sequencing on MCF-7-vector, ZEB1 and ZEB2 overexpressing cells. Analysis of total gene regulation using the NCI Pathway Interaction Database demonstrates an increase in genes associated with RhoA activity, an important mediator of cell motility, in ZEB2 overexpressing cells. However, the ZEB overexpressing cells show no change in morphology and canonical EMT markers remained unchanged between cell lines, suggesting a potential post-translational modification affecting ZEB1 and ZEB2 function. Together these results indicate that ZEB factors drive motility in breast cancer cells but are incapable of promoting a complete EMT in ER+ cells, warranting further investigation into the mechanisms involved in ZEB action. Elucidating the pathways involved in ZEB family function is an important step in understanding the processes underlying metastasis and has the potential to yield new therapeutic targets.
Acid-base disturbances have serious clinical consequences and are particularly critical in patients whose cardiopulmonary function is compromised. Cellular transport of NH$_3$ and NH$_4^+$ has important physiological significance in the regulation of acid-base balance. In the kidney, production and excretion of NH$_3$/NH$_4^+$ is critical for net acid excretion. Recently, two non-erythroid glycoproteins (Rhbg and Rhcg) belonging to the Rh family were suggested to be involved in NH$_3$/NH$_4^+$ transport. Thus far, the functional properties of these membrane proteins as transport mechanisms are not resolved. In our previous studies, we expressed Rh proteins in *Xenopus* oocytes and demonstrated that they transport both NH$_4^+$ and NH$_3$. As such, the Rh transporters are unique in being able to transport both the ionic and the neutral gaseous components of ammonia. Previous studies have shown that DIDS, a stilbene derivative known to inhibit anion exchangers, inhibits CO$_2$ transport by AQP1. This led us to hypothesize that DIDS might also inhibit transport of other gases such as NH$_3$ by Rh proteins. Selective inhibition of NH$_3$ transport by DIDS would indicate that NH$_3$ and NH$_4^+$ are transported by Rh glycoproteins through distinct pathways. We therefore conducted the present study to test the effects of DIDS on NH$_4^+$ and NH$_3$ transport by Rh glycoproteins. To do so we used ion-selective microelectrodes and two-electrode voltage clamp to measure changes in surface pH (pH$_s$) and whole cell currents (I) induced by NH$_3$/NH$_4^+$ and methyl ammonium (MA/MA$^+$) with or without DIDS. All experiments were conducted in *Xenopus* oocytes expressing Rhbg. Rhbg was expressed by injecting the oocytes with cRNA of the cloned gene. Control oocytes were injected with H$_2$O.

Our results indicate that in oocytes expressing Rhbg, exposure to 5mM NH$_4$Cl (NH$_3$/NH$_4^+$) caused a decrease in surface pH (pH$_s$) by 0.85±0.12 and an inward current of -58±10 nA. The decrease in pH$_s$ is caused by NH$_3$ influx whereas the inward current is due to electrogenic NH$_4^+$ influx. In the presence of DIDS, exposure to 5mM NH$_4$Cl caused a significantly smaller decrease in pH$_s$ (0.57±0.07) and current (-29±5 nA). The %inhibition of pH$_s$ and ΔI were 33% and 49%, respectively (P<0.05). Similarly, exposing oocytes expressing Rhbg to 5mM MA/MA$^+$ (a substitute to NH$_3$/NH$_4^+$) caused a decrease in pH$_s$ of 0.27±0.04 and an inward current of -29±6 nA. In the presence of DIDS, the MA/MA$^+$ induced changes in pH$_s$ (0.17±0.03) and current (-11±2 nA) were also inhibited (37% and 63%, respectively; P<0.05). DIDS had no effect on NH$_3$/NH$_4^+$ transport in H$_2$O-injected oocytes (not expressing Rhbg).

Our data indicate that 1) DIDS partially inhibits the transport of NH$_3$ and MA by Rhbg without affecting endogenous NH$_3$ and MA transport. 2) DIDS also inhibits the electrogenic transport of NH$_4^+$ and MA$^+$ by Rhbg. 3) DIDS is the only inhibitor shown to block both gas (NH$_3$) and ionic (NH$_4^+$) transport by Rhbg.

This work was supported by VA Merit Grant and Tulane Institutional Grant.
A generalized sparse regression model with adjustment of pedigree structure for variant detection from next generation sequencing data
Shaolong Cao, Huaizhen Qin, Hong-Wen Deng and Yu-Ping Wang

Complex diseases and traits are likely to be explained by both genotypes (e.g., common and rare genetic variants or SNPs) and environmental factors. Many association methods have been developed for detecting rare or common variants, and usually consider family design and unrelated individual design separately. To overcome the limitations of these methods, we develop a sparse regression model with the adjustment of pedigree structure and the incorporation of prior information. According to the pedigree’s impact on continuous phenotypes, we propose a modified Kinship matrix to adjust the correlation between pedigrees. To incorporate prior knowledge, we regularize the model with weighted penalty terms. To get the sparse solution, we evaluate and implement a fast threshold algorithm for solving the regression model with $L_{1/2}$ norm regularization. We also use the smooth gradient algorithm to solve the sparse model penalized with $L_p$ ($0 < p < 1$) regularization term. After getting the solution path, we use the AIC (Akaike Information Criteria) and stability selection hybrid methods to determine the sparsity level.

To evaluate our methods, we compare our method with the single marker test ($\chi^2$ test), Elastic-net, OMP (i.e., Orthogonal Matching Pursuit) and FOCUSS (i.e., FOcal Underdetermined System Solver).

To validate the results, we use the Encyclopedia of DNA Elements (ENCODE) data to simulate the different pedigree structures and test our methods on the Genetic Analysis Workshop 17 and 18 data. The results on both simulation and real data analysis show that our proposed sparse regression models can discover more true causal variants while maintain a lower false discovery rate. In addition, the models tend to detect common and rare variants evenly; the detection of true causal rare variants is not overwhelmed by unrelated common variants.

In conclusion, our proposed approach has the following advantages: (i) The model can adjust pedigree structures; (ii) The $L_p$ ($0 < p < 1$) norm regularization model can yield higher true positive rate while lower false discovery rate than other methods; (iii) The weighted regularization term provides a flexible way to incorporate prior knowledge; (iv) Our model can be easily extended to accommodate environmental covariates.
HUMAN DNA POLYMERASE β’s ENDONUCLEASE ACTIVITY PREVENTS MITOTIC CHROMOSOME INSTABILITY

Carron EC*, Chen D**, Makridakis NM*

*Tulane Cancer Center and Department of Epidemiology, Tulane University, New Orleans, LA, **Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

Most tumors have numerical and structural chromosome aberrations that can provide selective growth advantages. It has been reported that overexpression of DNA Polymerase B (Pol β) contributes to chromosome instability by inducing aneuploidy, telomere fusions, and a deficient mitotic checkpoint. However, the mechanism is currently unknown. Here, we report that Pol β’s endonuclease activity plays an important role in maintaining chromosome stability by collaborating with Topoisomerase IIα. Our results indicate that Pol β has endonuclease activity, and that this activity is required to prevent chromosomal instability during mitosis as deletion of Pol β induces aneuploidy, the formation of anaphase bridges, and micronuclei. Moreover, cells expressing the endonuclease deficient E216K mutant accumulate more anaphase bridges and micronuclei than those expressing wild-type Pol β. Consistent with these observations, Pol β localizes near the centromere in metaphase. This data suggests that Pol β’s endonuclease activity plays a role in mitotic chromosome segregation.

This work was supported by DOD grant PC094628 and NIH grant 8 P20 GM103518 (to NM).
IMPAIRED TYPE 1 INTERFERON SIGNALING IN CHRONIC LIVER DISEASE

Chandra PK1, Gunduz F1,2, Kurt R1,2, Hazari S1, Poat B1, Bruce D2, Cohen AJ3, Behorquez HE3, Carmody I3, Loss G3, Shores N2, Wu T1, Balart LA2, and Dash S1,2

1Pathology and Laboratory Medicine, 2Department of Medicine, Gastroenterology and Hepatology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA-70112; 3Transplant Surgery Section, Ochsner Medical Center, New Orleans, LA, USA. * Presenter

Background: Recently, we reported that persistently HCV infected culture induce endoplasmic reticulum (ER) stress and autophagy response that selectively down regulated the type I interferon (IFN-α) receptor (IFNAR1) leading to the IFN-α resistance (Am J Pathol 2014, 184: 214-229). The contributions of ER stress and autophagy response on the expression of IFNAR1 in HCV infected chronic liver disease are unknown. Aim: To investigate whether an increase in the ER stress and autophagy response is also associated with the reduced expression of IFNAR1 in HCV infected chronic liver disease are unknown. Methods: PHH were infected with cell culture grown HCV particles (HCV genotype 2a) and HCV replication was confirmed by the detection of viral RNA by real-time RT-PCR and HCV-core protein expression by Western blotting. A total of twelve liver biopsy specimens and nine explant livers from HCV infected CLD patients were included in this study. ER-stress and autophagy response and the level of IFN receptors in HCV infected PHH and CLD patients were measured by Western blotting. Result: HCV infection of PHH showed down regulated expression of IFNAR1 (Type I IFN-α receptor), IFNγR1 (Type II IFN-γ receptor) but not IL10Rβ (Type III IFN-λ receptor). Furthermore, reduced expression of IFNAR1 was also observed in HCV infected liver biopsy specimens and explants liver tissues with or without etiology of HCV infection. ER stress (by measuring BiP, IRE1α and peIF2α protein levels) and autophagy response (by measuring LC3II, Beclin 1 and ATG5 protein levels) were induced in HCV infected PHH and CLD patients. We observed that the activity of the mammalian target of rapamycin complex 1 (mTORC1) was inhibited both in HCV infected PHH and CLD patients. We also analyzed the upstream activity of mTORC1, and we found that both Akt and PI3K class I (phosphoinositide 3-kinase) was inhibited in HCV infected PHH and CLD patients. Conclusion: Our results verified the induction of ER stress and autophagy response in HCV infected primary human hepatocytes and chronic liver disease of viral and non-viral etiology. The expression of IFNAR1 is impaired in chronic liver disease due to ER-stress and autophagy response. Information from HCV infected PHH, biopsy specimen and explant liver tissues from CLD patients indicate that HCV induces autophagy through PI3K-Akt-mTOR pathway.
RETROSPECTIVE ANALYSIS OF PATIENTS WITH RENAL INVOLVEMENT IN MULTIPLE MYELOMA

Cossich SJ*, Khan AM*, Safah H**, Simon EE*** and Batuman V***

Department of Medicine, Section of Nephrology & Hypertension, Tulane University, School of Medicine, New Orleans, LA. Southeast Louisiana Veterans Health Care System, New Orleans, LA.

Purpose: Kidney involvement is a major and common complication of multiple myeloma (MM) caused by toxic effects of monoclonal free light chains (FLC) on renal proximal tubule cells. Reduction in serum FLC correlates with renal recovery, and MM patient survival. The main objective was to review the natural history of myeloma and the effects on kidney function in light of newer treatment protocols. We evaluated the clinical spectrum of patients with renal involvement in our MM program.

Methods: A retrospective chart review was conducted at the Tulane University Cancer Center in New Orleans, Louisiana. Patients older than 18 years both male and female were included. Data on demographics, renal function, type of myeloma, treatment regimens and outcomes were recorded. The primary objective was to assess the effect of chemotherapy on renal function recovery and survival.

Results: Thirty-one patients were included in the analysis. Age range was 27-76 years (average 58 years), 58% male and 42% female. Fifty-eight percent of patients received a combined therapy including thalidomide or revlimid (TR group) and 35% received a regimen including velcade (V group). Forty-five percent had IgG-κ type myeloma followed by IgG-λ and IgA-κ (16% each) myeloma. After six months of follow up, there was a significant improvement in eGFR in TR group ($p = 0.037$) and notable recovery in V group ($p = 0.209$) compared to their levels at presentation. Serum creatinine levels were also reduced in both TR ($p = 0.073$) and V ($p = 0.125$) groups compared to their levels at presentation. There was also a significant decrease in total protein at follow up in TR group ($p = 0.019$). In myeloma type, IgG-κ group demonstrated reduced serum creatinine ($p = 0.099$), a significant increase in eGFR ($p = 0.03$) and a significant decrease in total serum protein ($p = 0.035$) at follow up. In IgA-κ group, serum creatinine was reduced and eGFR was improved, but these improvements did not reach statistical significance. There was no notable improvement in the IgG-λ group at follow up. 24% ($n = 7/29$) of patients had eGFR $\leq 60$ ml/min before treatment and 20% ($n = 5/25$) after treatment. No patient required dialysis.

Conclusions: (1) Both therapeutic groups (TR and V) showed improvement in renal function and TR group was more effective than V group. (2) IgG-κ is the more common type of myeloma in patients with kidney involvement. (3) Renal outcomes were generally good in patients treated with these newer regimens and the prognosis of patients with myeloma kidney appears better than with the older regimens.
Aplasia of the cerebellum with holoprosencephaly and cutis laxa associated with an intronic deletion in NRG3

Crandall RO*, Ramalingam A*, Scott K*, Janssen A*, Kozicz T*, Chen TJ*, Morava E*

*Tulane University School of Medicine, Hayward Genetics Center, New Orleans, LA

Neuregulins are cell-cell signaling molecules involved in embryologic development that act as ligands to receptor tyrosine kinases. NRG3, encoding neuregulin 3, is located on the long arm of chromosome 10 between two low copy repeats (LCRs), LCR3 and LCR4, which have been reported to be involved in frequent and recurrent duplications and deletions. The product of proteolytic cleavage of NRG3 on the extracellular side of the plasma membrane is released into blood and eventually binds to Erb4. Previous reports suggest that mammalian NRG3 is expressed mainly in developing and adult brain and mammary epithelium, while expression profiles infer expression in skin and muscle. Transgenic mice that express NRG3 in the basal layer of epidermis are hairless and have thick, pale and wrinkled skin. Herein we report a newborn baby girl born at term with alobar holoprosencephaly, monoventricles, cerebellar agenesis, and cutis laxa. The patient had a motor developmental delay and feeding problems but no other organ involvement, including normal cardiac, pulmonary and renal function and no eye abnormalities. Detailed metabolic studies including cholesterol synthesis, protein glycosylation and SHH sequencing was normal. Elastin and collagen staining was normal despite skin histology showing epidermal thickening, increased wrinkling, decreased amount of body hair and increased pigmentation. Microarray CGH analysis of the patient's DNA revealed a 34.7 kb deletion in intron 3 of NRG3 located at 10q23.1 between LCRs 3 and 4. Staining in fibroblasts cells for cellular proliferation marker Ki67 revealed an increased rate of proliferation of NRG3-deficient cells compared to control cells. Two of the previously reported patients with deletions involving the chromosomal locus 10q22q23 showed severe developmental brain anomalies including retro-cerebellar cyst, hypoplastic cerebellum, ventriculomegaly and Chiari-I malformation. Until now no intronic deletion in NRG3 has been reported in patients in association with clinical symptoms. The clinical significance of the NRG3 intronic deletion found in our patient is not yet clear; however, the CNS and skin anomalies coupled with the expression pattern of NRG3 suggest a pathogenic function. This warrants further studies including identification of splicing and unmasked mutations, gene expression analysis, and elucidation of the role of regulatory factors present within the deleted intronic region.
A MICROENGINEERED 3D SENSORY NERVE MODEL TO ADVANCE DRUG DISCOVERY

Curley, JL*, Huval RM*, Miller OH**, Fan Y*, Hall BJ** and Moore MJ*

Depts. of *Biomedical Engineering and **Cell & Molecular Biology, Tulane University, New Orleans, LA

The concept of using 3D cultures and microscale engineered tissues as benchtop models for toxicity screening and drug discovery has rapidly been gaining ground in recent years. This concept has been exploited fairly successfully for epithelial and metabolic tissues, where metabolites and other soluble analytes constitute the measurable read-outs. For peripheral neural tissue, where bioelectrical conduction over long distances may arguably be the most appropriate physiological read-out, application of engineered tissue for testing has been lagging. Our objective was to develop an organotypic, micro-physiological tissue model that mimics the morphology of peripheral nerve tissue and that supports physiological measurements analogous to clinical nerve conduction tests.

Wide-field fluorescence, confocal, and TEM imaging revealed microengineered neural fiber tracts growing within the 3D volume of the Puramatrix hydrogel. The 3D constructs displayed relatively high degrees of parallel fiber growth, high density, and fasciculation. Field potential recordings show population responses with consistent delay, amplitude, and envelope, even under high frequency (100 Hz) stimulation, and data strongly suggest that the observed population spikes are wholly compound action potentials propagating down the microengineered neural fiber tract. Together, our data suggest that we can microengineer neural fiber tracts that resemble the morphology, physiology, and pharmacological responses of peripheral sensory nerve tissue.

Results demonstrate the feasibility of developing benchtop physiological models for drug testing and discovery. Development of a model that is truly analogous to clinical physiological assessments, however, will likely require tissue constructs that are myelinated, with large fiber diameters, and that are amenable to more rapid physiological assessments.

This work was supported in part by NSF CAREER (CBET-1055990) and DoD (W82XWH-12-1-0246).
Multiple epiphyseal dysplasia (MED) is a relatively common chondrodysplasia characterized by delayed and irregular ossification of epiphyses and early-onset osteoarthritis (OA). We and others have shown that this clinically and genetically highly heterogeneous disorder is caused by mutations in six genes: \textit{COMP} (for cartilage oligomeric matrix protein), \textit{COL9A1}, \textit{COL9A2}, \textit{COL9A3} (for collagen IX), \textit{MATN3} (for matrilin-3), and in \textit{SLC26A2} (solute carrier family 26, member 2 gene). However, mutations in these genes only explain approximately 50% of all cases, which indicates that defects in other genes are involved. Currently, the overlapping MED/OA phenotype is being reinvestigated since it may provide new insights towards understanding the genetic basis for common forms of osteoarthritis. New MED candidate genes and mutations in these genes may contribute to the development of common forms of osteoarthritis. We identified a new locus for MED using genome-wide scan and linkage analysis in MED families for whom we have previously excluded all currently known candidate genes. We use whole exome-sequencing to identify a new gene, which defect causes overlapping MED/OA phenotype. Whole-exome sequencing was performed by using a semiconductor technology introduced by Ion Torrent and utilized in next-generation sequencing system Ion Proton. Data analysis from whole-exome sequencing was conducted with a special emphasis on the interval with a high lod score (5.45). We plan to determine if changes in the new MED candidate gene may predispose to primary OA, by sequencing of the new candidate gene in Louisiana patients with primary knee osteoarthritis. Osteoarthritis affects more than 27 million people in the United States and there is no fundamental treatment for this disorder. Age and genetic factors strongly contribute to the development of primary form of osteoarthritis, for which the direct cause is still unknown.

This research was supported by: Louisiana Clinical and Translational Science (LA CaTS) Center as a Pilot Project and Bone and Joint Initiative USA Scholarship for Young Investigators to M.C.-R.; NIH (NHGRI, National Human Genome Research Institute) grant No. R25HG006110 to H.T., Department of Biostatistics, University of Alabama at Birmingham.

The whole-exome sequencing was conducted in the COBRE Genomics and Biostatistics Core at the Center for Aging, Tulane School of Medicine. Ion Proton system utilized in whole-exome sequencing was funded by NIH: NIGMS COBRE grant No. P20GM103629 to S. M. Jazwinski, Tulane Center for Aging, Tulane School of Medicine.
Insulin-like growth factor I (IGF-1) stimulates cell growth and proliferation. As we have shown in previous studies, systemic IGF-1 infusion reduces atherosclerosis in Apoe-null mice. To study cell-specific IGF-1 effects on atherosclerosis, we generated Apoe-null mice with smooth muscle cell (SMC)-specific promoter-driven IGF-1 receptor deficiency (22KI mice). We found that 22KI mice have a >90% reduction in aortic expression of IGF-1 receptor compared to FIR (control) mice (immunoblotting with a/IGF-1 receptor a/b) demonstrating that the IGF-1 receptor was successfully knocked out. 22KI mice have a reduced body weight (15% decrease, \( p<0.05 \)) and a disproportionately large decrease in size of SMC-rich organs such as the urinary bladder (37% decrease, \( p<0.05 \)) and the aorta (18% decrease, \( p=0.08 \)). 22KI mice have a marked decrease in SMC-positive aortic wall thickness (40% decrease, immunohistochemistry with a-smooth muscle actin, \( p<0.001 \) vs. FIR mice). 22KI mice have a similar number of SMCs in aorta cross-sections compared to control mice, however the individual SMCs in 22KI mice have a decrease in size (23% reduction, \( p<0.05 \)) suggesting that a SMC-specific IGF-1 receptor deficiency suppresses SMC growth but has no effect on SMC proliferation.

To study SMCs IGF-1 effect on atherosclerosis, 22KI and FIR mice were fed normal chow or a pro-atherogenic Western-type diet for 12 weeks. Next aortic atherosclerotic lesion surface area, aortic valve lesion cross-sectional area and plaque necrotic core size were quantified. 22KI mice fed a Western diet show increased lesion surface area (26±5%, 22KI vs. 7±4%, FIR, \( p<0.01 \)), cross-sectional area (39±5%, 22KI vs. 24±3%, FIR, \( p<0.001 \)) and larger necrotic core (56±8%, 22KI vs. 45±6%, FIR, \( p<0.05 \)). 22KI mice kept on normal chow show a similar increase in lesion size and necrotic core vs. control mice.

In summary, SMC-specific IGF-1 receptor deficiency reduces mouse body weight and organ size via suppression of individual SMC growth. Mice with SMC-IGF-1 receptor deficiency have increased atherosclerotic plaque size in the heart and aorta. Plaque in 22KI mice have elevated necrotic core per lesion suggesting an unstable plaque phenotype. As evidenced by our research, the development of SMCs and the stability of atherosclerotic plaques are both decreased in mice with the SMC-IGF-1 receptor deficiency.

This work was supported by grant from NIH/NHLBI (5-R01-HL070241-10).
DERMATOFIBROSARCOMA PROTUBERANS: EPIDEMIOLOGY AND PROGNOSTIC FACTORS

Phuong-Thanh Dang, BS¹, Ryan R. Riahi, MD², Diane Trieu, MD³, Nguyen D. Dang, MD⁴, Gaston A. de la Bretonne, MD²

¹Tulane School of Medicine, New Orleans, Louisiana, USA
²Department of Dermatology, Louisiana State University, New Orleans, Louisiana, USA
³Department of Dermatology, Tulane School of Medicine, New Orleans, Louisiana, USA
⁴Department of Radiation Oncology, Baylor College of Medicine, Houston, Texas, USA

Abstract:
The purpose of this study is to provide prognostic factors and survival rates for a rare tumor, dermatofibrosarcoma protuberans, using a large population data collected by the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute.

Introduction: Dermatofibrosarcoma protuberans (DFSP) is a rare slowly growing soft tissue tumor that can be locally aggressive but infrequently metastasizes. The tumor often presents as an asymptomatic, solitary, indurated plaque that may be flesh colored, red-brown, or violaceous. It is most commonly diagnosed in young to middle aged adults, between the ages of 20 and 50. DFSP has an almost equal sex distribution with a slight male predominance. It occurs most commonly on the trunk, followed by the proximal extremities, and the head and neck area.

Methods and Materials: Data from 7194 cases of DFSP were obtained from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute from 1973 to 2009. The JMP 10 statistical software (SAS Institute, Inc.) was used to compare the difference in patient and tumor characteristics. Data on age, extent of disease, histological grade, lymph
node involvement, primary site, race, radiation therapy, sex, survival, and surgical type were investigated. Estimates of overall survival were performed using the Kaplan-Meier method. The log-rank test provided estimates of statistical significance during multivariate analysis.

**Results:** There was a predominantly higher incidence in Caucasians (71%). In addition, there was a higher incidence among women (53%) than men (47%). The overall survival (OS) rates at 10 years and 20 years were 89.8% and 79.9%, respectively. Women had a slightly higher OS rate at both 10 years and 20 years than men (92.2% and 82.4% vs. 86.5% and 76.9%, respectively, p-value <0.01). The majority of the cases (80%) did not involve either lymph node or distant metastasis. The 10-year and 20-year OS rates of tumors with distant metastasis compared to tumors without metastasis are 44% and 0% vs. 90.3% and 80.5%, respectively (p-value <0.0001). Tumors >5 cm had a worse prognosis than smaller tumors ≤5 cm with 10-year OS rates of 83.4% vs. 90%, respectively. Those who received local tumor destruction/excision had a 10-year OS rate of 88.5% compared to 91.4% for those that received wide/radical excision.

**Conclusion:** DFSP is a rare malignancy that infrequently metastasizes. It mainly occurs among Caucasians more so than any other race. It most frequently occurs on the trunk followed by the extremities.

This work was not supported by any grant. The authors have no disclosure.
ASSESSMENT OF PRO-INFLAMMATORY (MSC1) AND ANTI-INFLAMMATORY (MSC2) HUMAN MESENCHYMAL STEM CELL PHENOTYPES GROWN ON SYNTHETIC ABSORBABLE BIOMATERIALS FOR REGENERATING ESOPHAGEAL TISSUE

Dashti DC*, Johnson J**, Abdulnour-Nakhoul S***, and Betancourt AM****

* Bioinnovation (IGERT) Program, Tulane University, New Orleans, LA
** Nanofiber Solutions, LLC
*** Departments of Medicine and Physiology, Tulane University, New Orleans, LA
**** Department of Medicine, Tulane Center for Stem Cell Research & Regenerative Medicine, Tulane University, New Orleans, LA

Esophageal cancer is the ninth most complicated gastrointestinal malignancy, and every year 17,990 new cases and 15,210 deaths occur annually in the United States. The current treatment for serious malignancies of the esophagus such as esophageal cancer is an esophagectomy, which is a surgical procedure to remove the affected tissue of the esophagus and splice the lower intestines on to the remaining tissue. Commonly after this procedure, patients cannot swallow and have to rely on feeding tubes for nutrition. Subsequently, the quality of life after an esophagectomy is low and morbidity rates are high. Our solution for esophageal resection is to develop an absorbable synthetic biomaterial in-vitro seeded with bone marrow multipotent stromal cells, commonly referred to as mesenchymal stem cells (MSCs), to regenerate a portion of the esophagus in-vivo.

Significantly, MSCs are known to promote tissue healing and regeneration. In recent clinical studies, biodegradable scaffold tubes seeded with MSCs successfully formed functional tracheas when implanted into patients. In this study, we plan to develop similar tube scaffolds seeded with MSCs that can be used as implantable esophageal tubes. By seeding the pro-inflammatory MSC1 and anti-inflammatory MSC2 phenotypes onto a synthetic absorbable biomaterial, it is hypothesized that robust wound healing/regeneration of the esophagus will occur. Nevertheless, for the MSCs to help regenerate esophageal tissue in-vivo, proper integration of the cells with a selected biomaterial is crucial. In-vitro studies of cellular adhesion, proliferation, and viability on the biomaterials must be assessed. Importantly, the synthetic biomaterial must also exhibit similar biomechanics like that of esophageal tissue for proper regeneration in-vivo. Such biomaterials of consideration are PDO and PCL (Polydioxanone and Polycaprolactone). Overall, this work will characterize MSC1 and MSC2 phenotypes on different absorbable synthetic biomaterials such as PDO and PCL for future therapeutic use to regenerate an excised esophagus.

Funding was provided by the National Institutes of Health 1P20RR20152-01, 1R43AR061902, Department of Defense OC073102 Concept Award and research support from the Tulane Cancer Center and the Center for Stem Cell Research and Regenerative Medicine.
MEDICAL STUDENT SENSITIVITY TRAINING: CHANGING ATTITUDES TOWARD WOMEN WITH SUBSTANCE USE DISORDER

Delaney CM*, Robinson WR*

*Department of Obstetrics and Gynecology, Tulane University School of Medicine, New Orleans, Louisiana, US

Negative attitudes by healthcare providers toward patients with substance use disorder (SUD) are common and harmfully affect patients’ medical compliance, understanding of personal health conditions, and future utilization of health services. Medical students will have a role in caring for men and women with SUD throughout their medical education and their careers. Past studies have not addressed how educational interventions for medical students can improve their understanding of patients with SUD. This project tackles medical students’ perception of patients with SUD by looking at patient centered outcomes and students’ knowledge and attitudes toward this population. Specifically, the project focuses on an interventional training video for medical student volunteers at one of Tulane University School of Medicine’s student-run clinics that serves women in rehabilitation for substance dependence. A pre-intervention and post-intervention survey will be given to all students in the current and upcoming first-year classes. These surveys will measure differences in knowledge and attitudes concerning addiction and SUD, between students that participate in the intervention and those that do not. Additionally, patient satisfaction surveys will be administered to clinic patients in order to document changes in patient care before and after the intervention. Our hypothesis is that the intervention will positively impact patient care and medical student understanding by improving the communication between medical students and patients with SUD, while creating an environment that contextualizes these patients’ conditions. The results from this project will create an outline for future curriculum and training of medical students to work with patients with SUD.
Deskin Brian J*, Zhuang Yan*, Lasky Joseph A*, Shan Bin*,**

Notch Signaling and TGFβ-1: HDAC6 mediation of converging pathways in A549 cells

From *Department of Medicine and **Tulane Cancer Center, Tulane University Health Sciences Center, New Orleans, LA 70112

The purpose of this study is to investigate the role HDAC6 plays in modulating the TGFβ-Notch signaling axis when activated by TGFβ-1 in a cell culture model of NSCLC. We hypothesize that HDAC6 is necessary for the TGF-β-Notch signaling axis and that inhibition of HDAC6 abrogates this signaling cascade. Using the human adenocarcinoma lung epithelial cell line, A549, the TGFβ-1 cytokine was used to induce an epithelial-mesenchymal transition phenotype. Inhibition of HDAC6 enzymatic activity was achieved using the small molecule inhibitor, tubacin. Genetic knockdown of HDAC6 expression was accomplished by two different approaches: stably transduced cell lines were established using a lentiviral construct and also by an HDAC6-targeted siRNA transfection approach. Microtubule stabilizing agents to investigate the regulatory role HDAC6 plays on microtubule dynamics while an HSP90 inhibitor, 17AAG, was used to investigate the HDAC6-mediated HSP90 functions. Canonical Notch signaling was blocked using the γ-secretase inhibitor DAPT and with siRNA directed against Notch1. In this study we show that pharmacological inhibition and small interfering RNA against HDAC6 attenuates activation of Notch-1 downstream target genes both at the transcriptional level and the protein level; similarly, pharmacological inhibition of the γ-secretase complex results in attenuated expression of notch target genes. Our findings indicate a novel function of HDAC6 mediating the TGFβ-Notch signaling axis, further implicating HDAC6 as a key regulator of EMT, making it an ever-more attractive therapeutic target for protection against tumorigenesis and metastasis in NSCLC.
The involvement of circadian clock genes in tumor necrosis factor alpha (TNFα) and transforming growth factor-beta1 (TGF-β1)-induced lung injury and fibrosis

Dong C*, Luo F*, de Cabo R**, Lasky JA*, Sanchez C*

*Department of Medicine, Section of Pulmonary Disease and Critical Care, Tulane University School of Medicine, New Orleans, LA

**Laboratory of Experimental Gerontology, NIA, NIH, Baltimore, MD

Background: Circadian clock regulators are involved in the modulation of proliferation, autophagy, metabolism, and cell survival. Like most organs, lung exhibits robust circadian rhythm and several genes critical for lung maintenance and repair have been found to show circadian-like oscillation in expression. However, the role of circadian clock genes in lung injury and fibrosis is still largely unknown. We hypothesize that aging and lung injury is accompanied by deregulation of peripheral circadian clock genes. Results: In this study, we found a severe deregulation of genes involved in circadian clock circuitry in lungs from aged mice compared to young mice, as well as TNF-transgenic and TGF-β1-adenovirus-infected mice compared to their controls. In other words, these injury models recapitulated the expression changes of circadian clock genes. The amplitude of clock genes was deregulated by TNFα and TGF-β1 in lung epithelial cells. Meanwhile, knockdown of key regulators of circadian clock significantly attenuated TGF-β1 and TNFα-induced MMP9 expression and altered TGF-β1-induced expression of Epithelial-Mesenchymal Transition markers in lung epithelial cells. Conclusions: Our results demonstrate that TNFα and TGF-β1 simulate aging in the effect on expression of circadian clock genes. TNFα and TGF-β1 affect the expression of core circadian clock genes in lung epithelial cells. On the other hand, knockdown of circadian clock genes interferes with TNFα and TGF-β1-induced signaling transduction and activities in lung epithelial cells. Future directions: The relevance of gene expression changes of the circadian clock genes during lung aging and disease will be further studied using in vivo animal models.
GROWING UP OR GROWING OLD? CELLULAR AGING LINKED WITH TESTOSTERONE REACTIVITY TO STRESS IN YOUTH


*Department of Psychiatry, Tulane School of Medicine
** Department of Psychology, University of New Orleans
***Department of Global Community Health, Tulane School of Public Health

Objectives: There are wide individual differences in rates of aging, with adversity advancing aging even within young populations. Given the established relation between testosterone and aging in older adults, we tested whether buccal cell DNA telomere length (bTL), an established cellular biomarker of aging, was associated with testosterone in youth, including stress-reactive, diurnal and basal salivary testosterone.

Methods: This study was approved by the Tulane University Institutional Review Board. Youth, age 5-15 years, were recruited from greater New Orleans, Louisiana. Families were recruited using street outreach techniques, including ethnographic mapping and targeted sampling and through schools in these communities. Testosterone reactivity or diurnal rhythms and bTL were available on 77 children.

DNA was collected using Isohelix SK1 buccal swabs (Cell Projects, Kent, UK) and extracted using the QIAamp® DNA mini kit protocol (Qiagen, Valencia, CA, USA). Concentration of extracted DNA was quantified with a Qubit dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA). Average relative bTL was determined from the telomere repeat copy number to single gene (albumin) copy number (T/S) ratio using an adapted monochrome multiplex quantitative real-time PCR (MMQ-PCR) and a BioRad CFX96.

Saliva was collected before, immediately after, 20-min and 60-min post TSST-C to capture stress reactivity and recovery. All sessions began in the early afternoon after a 30-min rest period. Across two days, saliva was collected upon awakening, 30-min later, in the early afternoon and at bedtime to capture waking basal levels and testosterone’s diurnal rhythm. Testosterone was assayed using a commercially-available enzymeimmunoassay (Salimetrics, PA). Hierarchical linear modeling then captured testosterone “best estimates” as Empirical Bayes estimates of each youth’s testosterone level, reactivity, recovery and diurnal rhythm score.

To account for correlation between siblings or children living in the same household and ensure correlated data did not inflate findings, generalized estimating equations (GEE) analyses were employed. All analyses controlled for child sex, age, body mass index (BMI), maternal and paternal age at conception, race, pubertal stage, and maternal education.

Results: A significant association between bTL and all three measures of acute testosterone activity was detected (Table 1). Specifically higher peak levels of testosterone, increased slope to peak and decreased slope to recovery were all significantly associated with shorter bTL, even when controlling for basal testosterone levels. No direct association was found between bTL and diurnal testosterone. A significant effect of sex was found on the relationship between bTL and testosterone levels. In boys
when controlling for basal testosterone, both acute testosterone levels and diurnal levels were significant, independent predictors of bTL. In girls, when controlling for basal testosterone neither acute testosterone nor am testosterone predicted bTL; however, pubertal timing became a significant predictor of bTL, where more advanced pubertal status was associated with shorter bTL (Table 2).

Conclusions: We found bTL was associated with greater testosterone reactivity, higher peak testosterone, and slower recovery from an acute social stressor in children, even after accounting for gender, age, parental age at conception, maternal highest educational achievement, and puberty. These findings begin to outline a complex integrated system that incorporates the gonadal axis as part of the biological mechanisms linking accelerated maturation and stress.
**Funding Source:**

The project described was supported by Award Number K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health & Human Development or the National Institutes of Health.
PRL-3: Novel Biomarker and Potential Therapeutic Target for Prostate Cancer Progression

Donna R. Edwards*, Kryszttof Moroz***, Astrid Engel****, Asim B. Abdel-Mageed**, Debasis Mondal*. Departments of Pharmacology*, Urology**, and Pathology***, Tulane University School of Medicine. Department of Epidemiology****, Tulane School of Public Health and Tropical Medicine

Phosphatases of Regenerating Liver (PRL), particularly PRL-3, have been designated as oncogenes because of their roles in promoting epithelial-to-mesenchymal transition (EMT) and metastasis in various cancer models (Liver, Colorectal, Ovarian, Breast, etc.). However, little is known about the role of PRL-3 in prostate cancer (PCa). We hypothesize that PRL-3 promotes PCa progression, and therefore, PRL-3 can be used as a novel biomarker and an effective therapeutic target against aggressive disease. Tumor sections (FFPE) stratified by Gleason Score (<6 or >8) and Race (African-American and Caucasian) were obtained from the Louisiana Cancer Research Consortium (LCRC) Biospecimen Core. We first investigated whether the expression and sub-cellular localization of PRL-3 is associated with PCa disease severity in patients. Immunofluorescence microscopy (IFM) analysis of tumor sections indicated that indeed PRL-3 expression is augmented in disease progression and its subcellular localization is associated with high-grade disease. In vitro studies were carried out using the androgen-dependent LNCaP cells and the castration-resistant PCa (CRPC) lines C42B and LNCAP-SF. Induction of androgen receptor (AR) signaling by R1881 exposure was seen to induce PRL-3 protein levels, and more interestingly, nuclear translocation. Furthermore, PRL-3 was constitutively expressed in the nucleus of CRPC cell lines. In order to determine functionality of PRL-3 in the nucleus, we performed subcellular fractionation studies and subsequent immunoblot analysis of CRPC cell lines exposed to R1881. Interestingly, we found that PRL-3 is tightly bound to chromatin in these cells lines. Therefore, we postulate that PRL-3 phosphatase activity may be a crucial link between AR signaling and the aggressive EMT phenotype in PCa cells. Preliminary shRNA studies have shown that PRL-3 affects cell survivability of CRPC cell lines. Co-immunoprecipitation assays validating the nuclear co-translocation of AR and PRL-3 are underway. This study is expected to establish PRL-3 as a novel biomarker for advanced prostate cancer and a potential therapeutic target.
SPECTRAL UNMIXING OF DUAL-FLUORESCENCE STAINING FOR POINT-OF-CARE PATHOLOGY

Elfer KN*, Wang M*, Kimbrell HZ** and Brown JQ*
*Department of Biomedical Engineering, Tulane University, New Orleans, LA, USA
**Department of Pathology and Laboratory Medicine, Tulane University, New Orleans, LA, USA

The identification of positive surface margins (PSM) in biopsy removal is time-critical to avoid further pain and suffering and prevent relapse. By using a dual fluorescent stain to mimic standard hematoxylin and eosin (H&E) staining, clinicians will have access to a time-efficient and non-invasive method for analyzing the surface of a biopsy. Presented here is the use of the nuclear stain, Proflavin, and the cytoplasmic stain, Eosin, to replicate H&E. Because these two stains have overlapping spectra (Figure A), the nucleic stain is indistinct from surrounding stroma. A spectral unmixing program is being developed to isolate the two fluorophores and subtract spectral overlap.

Two channels on a Nikon A1 confocal microscope are used to create a single combined image of the fluorescently stained tissue (Figure B). In Figure C, the two channels have been colored to identify Eosin (red) and Proflavin (Green) using ImageJ. Spectral unmixing uses reference spectra for each stain that can be compared to the mixed spectral image using the same imaging settings. By isolating the wavelengths where each fluorophore is strongest and correlating it to the pixel intensity of each channel, it is possible to approximate the contribution of each fluorophore to the pixel intensity. The algorithm combines the known spectral peak wavelengths, the reference data, and the original image to compute a new, higher contrast image. Using the algorithm in MATLAB Figure C is transformed to the image in Figure D.

**Figure A: Combined Spectra of proflavin and eosin.**
**Figure B: Stitched image of a 10um section of prostate tissue at 10x on a Nikon 1A confocal.**
**Figure C: Taken from Figure B within the red outline.** An enlarged and colorized image of the selected area. The two channels were used to color the image (red and green) without any other modification. **Figure D: The result of running the previous image through the MATLAB algorithm.** Pseudo-coloring was also then added to the image for contrast.

Improving the visible contrast between the nuclei and stroma will aid clinicians to quickly identify irregularities in the tissue that might indicate a PSM. The current algorithm requires constant modification to achieve high-contrast results, but further work is being done to automate the process. Additionally, work is being done to best select the specific wavelengths needed to isolate the nuclei and stroma. Validation for pathology will be conducted by comparing H&E stains of these sections against the fluorescent images by a trained pathologist.

This work was supported by NIH/NCI R21CA159936, Tulane University School of Science and Engineering, and the NSF Graduate Research Fellowship Program.
NEOPLASTIC REPROGRAMMING OF PATIENT-DERIVED ADIPOSE STEM CELLS BY PROSTATE CANCER CELL-ASSOCIATED EXOSOMES

Zakaria Y. Abd Elmageed1*, Yijun Yang1, Raju Thomas1,4, Manish Ranjan1, Debasis Mondal2,4, Krzysztof Moroz3,4, Zhide Fang5, Bashir M. Rezk1, Krishnarao Moparty1,6, Suresh C. Sikka1,2, Oliver Sartor1,4, Asim B. Abdel-Mageed1,2,4**

Departments of Urology1, Pharmacology2, Pathology3 and Tulane Cancer Center4, Tulane University Health Sciences Center; Biostatistics Program5, School of Public Health, Louisiana State University Health Sciences Center; and Department of Urology6, Southeast Louisiana Veterans Health Care System, New Orleans, Louisiana, USA, 70112

Background: Emerging evidence demonstrates that circulating mesenchymal stem cells (MSCs) are significantly higher in obese than lean cancer patients and are often recruited to tumor sites but their functional significance in tumor growth and disease progression remains elusive. Exosomes, small extracellular membrane-enclosed vesicles, are involved in cell-cell communications and modulation of cell biology, primarily through trafficking of genomic and proteomic materials into target recipient cells. The objective of this study is to investigate the role of prostate cancer (PCa) cell-derived exosomes in neoplastic transformation of PCa patients’ tumor-tropic MSCs.

Methods: Adipose tissue-derived stem cells (pASCs) were procured from obese PCa patients and their purity confirmed by FACS analysis. A transendothelial well system was used to enrich pASCs with high tumor homing potential. Exosomes were purified from castration resistant (C4-2B and PC-3) cells by differential ultracentrifugation and their purity was verified by cryo-transmission electron microscopy and PCR analysis. Induction of prostate tumors by pASCs primed with PCa-cell derived conditioned media (CM) or exosomes was examined in athymic nude mice. Tumor formation was verified by cytogenetic analysis and expression of epithelial, neoplastic, and vasculogenic markers by immunofluorescence analysis. Characterization of exosomes oncogenic “cargo” of PCa cells, including miRNAs, mRNAs and proteins was examined by miRNA array, qPCR and LC/MS-MS analyses, respectively.

Results: Herein we report that prostate cancer (PCa) cell microenvironment subverts PCa pASCs to undergo neoplastic transformation. Unlike normal ASCs, the pASCs primed with PCa CM formed prostate-like neoplastic lesions in vivo and reproduced aggressive tumors in secondary recipients. The pASC tumors acquired cytogenetic aberrations and mesenchymal-to-epithelial transition (MET) and expressed epithelial, neoplastic, and vasculogenic markers reminiscent of molecular features of PCa xenografts. Our mechanistic studies revealed that PCa cell-derived exosomes are sufficient to recapitulate formation of prostate tumorigenic mimicry generated by CM-primed pASCs in vivo. In addition to down-regulation of the large tumor suppressor homolog2 (Lats2) and the programmed cell death 4 (PDCD4), the tumorigenic reprogramming of pASCs was associated with trafficking by PCa cell-derived exosomes of oncogenic factors, including H-ras and K-ras transcripts, oncomiRNAs miR-125b, miR-130b, and miR-155 as well as the Ras superfamily of GTPases Rab1a, Rab1b, and Rab11a.

Conclusions: Our findings unravel a novel and previously uncharacterized role for PCa cell-derived exosomes in MET and clonal expansion of prostate tumors through neoplastic reprogramming of tumor-tropic ASCs. Targeting tumor-derived exosomes may represent a new therapeutic modality to circumvent metastatic tumors in cancer patients.

This work was partially supported by grants from the NIH/NCI (U01CA149204-01A1; A.B.A), ACS (RSGT-09-248-01-CCE; A.B.A), and two DoD grants (PC102056; A.B.A. and PC080811, D.M.).
Inhibition of HCV IRES translation by Type I and Type III interferon is impaired in human hepatoma cell lines with an unfavorable SNP rs12979860 of IL-28B gene

Pauline Ferraris¹, Nathan Shores², Luis Balart² and Srikanta Dash¹,²

¹Pathology and Laboratory Medicine, ²Department of Medicine, Gastroenterology and Hepatology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA-70112.

Research Program: Immunology, Infection & Inflammation

Introduction: Single nucleotide polymorphisms (SNPs) located within the IL-28B gene promoter (IFN-λ3) (rs12979860 CC or rs8099917 TT) was discovered to be a strong predictor for hepatitis C virus clearance by combination therapy of IFN-α, ribavirin and protease inhibitors. The mechanism how the IL-28B gene polymorphisms relate to HCV clearance is unclear.

Aim: To investigate the molecular mechanisms by which IL-28B gene promoter polymorphisms and the IFN-λ signaling play role at the regulation of HCV translation inhibition by interferons.

Methods: Ten different human hepatoma cell lines with different SNP rs12979860 of IL28B gene: three CC, three TT, four CT were transfected with either HCV IRES-luc or HCV IRES-GFP reporter plasmid using Fugene 6 method. Inhibition of HCV IRES after treatment with Type I (IFN-α), Type III (IL-29) and ribavirin was quantitatively measured. The activation of Jak-Stat pathway and various ISGs after IFN-treatment was investigated by Western blot analysis.

Results: Our results show that all three TT cell lines show significantly less inhibition of HCV IRES translation by IFN-α or IFN-λ, and express a higher IFN-λ1 protein level compared to the CC genotype cell line. The investigation of the Jak-Stat pathway induced by IFN-α, IFN-λ1 and IFN-λ3 treatment showed a high level of PKR in the TT cell line compared to the CC but the phosphorylation of PKR and eIF2α seems to be less induced in the TT cell lines which could explain the difference of HCV IRES translation inhibition between the two genotype.

Conclusion: These results suggest that the poor response to IFN-α/Ribavirin treatment among chronic HCV patients with unfavorable genotypes can be due to the impaired of HCV IRES translation inhibition by the IFN-λ signaling.
STAGING TOTAL THYROIDECTOMIES: DOES INTRAOPERATIVE NERVE MONITORING APPROPRIATELY PREDICT POST-OPERATIVE RECURRENT LARYNGEAL NERVE FUNCTION?

Fontenot TE*, Randolph GW**, Masoodi H***, Friedlander H*, Kandil E* ***
*Department of Otolaryngology, Tulane University School of Medicine, New Orleans, LA
**Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Boston, MA
***Department of Surgery, Tulane University School of Medicine, New Orleans, LA

Total thyroidectomy presents a significant risk of bilateral recurrent laryngeal nerve (RLN) injury, a life threatening complication. Traditionally, this risk has been managed through visual nerve identification with meticulous dissection along its course to the laryngeal entry point. However, stages of nerve praxia are inconsistently evident in a visually intact nerve. The practice of staging of a total thyroidectomy aims to preserve the functionality of the remaining RLN when injury is identified on the side of initial dissection. Intraoperative nerve monitoring (IONM) is gaining wide acceptance as a real-time indicator of changes in the nerve function. We hypothesize that IONM can provide reliable and appropriate feedback regarding functional status of the stimulated RLN during total thyroidectomy. We aim to examine the effectiveness of IONM technology in avoiding risk of bilateral RLN injuries by facilitating the decision to stage the surgical procedure.

Review of medical records identified ten procedures staged based on unfavorable signal change detected by IONM. Eight patients were found to have ipsilateral vocal cord paresis on post operative laryngeal examination (IONM positive predictive value 80%); the group had suffered an average drop of 68%. Three patients had no signal in response to stimulation. In the other five patients, the signal dropped by a mean of 49%.

In the present study, none of the patients experienced bilateral RLN injuries and IONM reliably indicated significant changes in the level of nerve function. A drop in amplitude by 49% in IONM signal and complete loss of signal were nearly equally predictive of post-operative RLN dysfunction. A few of the patients experienced apparent resurgence of signal, however, post-operative flexible laryngoscopy demonstrated vocal cord dysfunction and hoarseness. Post-operative glottic exam with determination of clear-cut laryngeal function is the gold standard neural outcome quality measure available after thyroidectomy. Among the 8 patients with documented vocal cord dysfunction, 3 (37.5%) were completely asymptomatic. These findings
suggest that incidence of RLN injury and consequently the true potential risk of bilateral RLN injury may be underestimated by groups that perform laryngoscopic evaluations only in the presence of symptoms.

IONM results accurately and reliably indicate post-operative ipsilateral vocal cord dysfunction suggesting that IONM is well suited for pivotal role in decision to stage thyroid surgery to reduce the risk of bilateral RLN injury. The era of nerve management through nerve visualization alone appears to be at an end given the functional dynamic importance of electrophysiological information afforded through neural monitoring.
DELIVERY OF THE CELL IMPERMEANT MUSHROOM TOXIN PHALLOIDIN VIA SPONTANEOUS MEMBRANE TRANSLOCATING PEPTIDES

Taylor Fuselier¹, William Wimley¹.

¹Tulane University, New Orleans, LA 70112, USA.

The cellular membrane exhibits a barrier which hinders the entry of many bioactive molecules. Several strategies exist to deliver impermeant bioactive molecules such as detergents, liposomes, electroporation, and pore-forming peptides. However, these methods rely on the physical disruption of the cellular membrane to deliver bioactive molecules and thus prove to be cytotoxic. Cell penetrating peptides (CPPs) are a unique family of peptides capable of crossing the lipid bilayer of cells. The mechanism of entry for many CPPs often relies on cellular energetics via endocytosis (i.e. HIV’s TAT peptide, Arg9, penetratin, etc.). Recently, our lab developed a high-throughput orthogonal screen capable of identifying spontaneous membrane translocating peptides (SMTPs) from a 10,000 peptide member library. TP2 (PLIY-LRLLRGQWC) was one of a handful of sequences identified in the screen as being able to translocate into synthetic vesicles as well as in vitro while conjugated to a cell impermeant fluorophore. Here we seek to explore the capabilities of TP2 to deliver an impermeant bioactive molecule, phalloidin, a toxin from the death cap mushroom Amanita phalloides.
Congenital disorders of glycosylation (CDGs) are phenotypically diverse genetic syndromes caused by impaired glycoprotein synthesis. Phosphoglucomutase 1 deficiency (PGM1-CDG) is a newly discovered CDG characterized by hypoglycemia, hepatopathy, muscle involvement and endocrine dysfunction. Intercellular cell adhesion molecule 1 (ICAM-1) serves as a useful biomarker for the measurement of cell surface glycosylation. Dietary galactose supplementation is a promising experimental therapy for PGM1-CDG. We present two 19-year-old females, Patients 1 and 2, with a history of recurrent hypoglycemic episodes, rhabdomyolysis and endocrine dysfunction diagnosed with PGM1-CDG. We performed a comprehensive metabolic panel and evaluated glycosylation of plasma secretory proteins. Fibroblasts from Patient 1 were cultured and stained for ICAM-1 to measure glycosylation. In vitro galactose complementation assays with repeat ICAM-1 staining were then performed on fibroblasts to assess for improved glycosylation. Patient 1 also received oral galactose supplementation therapy for one year. Patient 1 initially presented with hypoglycemia (lowest recorded value: 1.0 mM) and symptoms of exercise intolerance. Upon examination, Patient 1 was found to be below the fifth percentile for growth and to have a bifid uvula with a cleft palate and Pierre Robin syndrome. Patient 1 demonstrated hypogonadotropic hypogonadism, malignant hyperthermia and rhabdomyolysis with a maximum creatine kinase of 50,000 units/L. Patient 1 also exhibited dilated cardiomyopathy, hepatopathy, increased glycogen storage and steatosis. Patient 2 presented with severe rhabdomyolysis with myoglobinuria, hypoglycemia and hypogonadotropic hypogonadism. ICAM-1 staining in Patient 1 fibroblasts revealed markedly decreased glycosylation compared to a control cell line. Patient 1 fibroblasts were complemented with galactose at 0.25, 0.5, 0.75, and 1.0 mM concentrations for five days and subsequent ICAM-1 staining revealed a restoration of glycosylation. ICAM-1 staining improved with increasing galactose concentration through 0.75 mM, at which point it stayed constant despite increases in concentration. Following one year of oral galactose supplementation, Patient 1 demonstrated significant improvement in liver and endocrine function and her hypoglycemia resolved. In summary, both patients showed abnormal glycosylation and impaired glycogen synthesis but their different presentations demonstrate the phenotypic heterogeneity of PGM1-CDG. As galactose therapy appears to be a promising dietary intervention for normalizing protein glycosylation, future directions include targeted screening for PGM1-CDG and a clinical trial to evaluate the success of oral galactose supplementation.

This work was supported by The Hayward Foundation.
Title: BLEEDING THE DETAILS: ACUITY AND MORTALITY YIELD SMALL MOLECULE BIOMARKERS OF LASSA FEVER INFECTION

Authors: Gale, T.V., Horton, T.M., Garry, R.F.

Abstract: Unique amongst the swift killing Viral Hemorrhagic Fevers, Lassa virus annually afflicts 300,000 to 500,000 people in central and western Africa, debilitating patients with few treatment options and rapid diagnosis paramount to positive outcome where 50-70% of patients face terminal disease. While modern Lassa Fever (LF) diagnostics work admirably, caveats surrounding delay and variation in primary immunoglobulin response and time to detection coupled to immunoglobulin half-life (persistence) provide impetus for sensitive diagnostics development. Exploitation of fundamental physiological changes resultant from early viral infection/replication will delineate febrile illness of early, acute LF and illness of non-LF etiology. Here we investigate differences at the serum small molecule level in LF patients compared against non-LF and convalescent LF patients. Patient sera were collected from endemic locales; the small molecule fraction extracted, and detected using Ultra High Performance Liquid Chromatography Mass Spectrometry. Group-to-group metabolite disparities where analyzed through metabolomics software and machine learning to detect, scrutinize, and elucidate features possessing strong diagnostic and prognostic utility for seropositive LF. Metabolomes of acute and terminal LF patients are dramatically altered when compared to controls. Dysregulation of pathways mediating amino acid, nucleic acid, hemoglobin, and lipid metabolism where detected through disparity in absolute concentration of blood-borne metabolites. Features with putative identification such as phosphohydroxypropyruvic acid, 7-methylnosine, I-Urobilin, and palmitoylcarnitine offer examples of biomarkers with a range of correlative strength while additional yet-to-be identified features with strong biomarker character are detected. Loss of soluble fibrin monomers is significant in hemorrhagic fever patients. The Random Forest machine learning algorithm has provided the strongest biomarker candidates statically qualified for further characterization. This investigation has produced multiple serum analytes that when used singularly or synergistically with binary outcome, produce a high accuracy diagnostic method with exceedingly desirable sensitivity, specificity, and ROC and favorable prognostic indication. Chemical analytics, method development, and clinical evaluation will be required to verify the recorded observations and determine the applicability of a serum small molecule diagnostic and/or prognostic approach to VHF.
UNCONVENTIONAL VIEW ON GABAERGIC AND GLYCINERGIC INHIBITION OF RVLM NEURONS

Gao H, Derbenev AV.*

*Department of Physiology, Tulane University Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112 USA

**Neuroscience program, Tulane University

The rostral ventrolateral medulla (RVLM) is an important integrative center in the sympathetic nervous system regulating homeostatic functions including cardiovascular activities. The RVLM activity is largely mediated by GABAergic inhibition that can influence cardiovascular function. Glycine receptors (GlyRs) are also involved in the regulation of the RVLM activity, and they are intensely expressed in the RVLM. In this study, we used the transsynaptic retrograde viral tracer PRV-152 to identify kidney-related neurons in the RVLM and examined inhibitory mechanisms mediated by GABA\(_A\) receptors and GlyRs by using whole-cell patch-clamp recordings. Application of GABA (100\(\mu\)M) increased the tonic current and decreased the phasic current. In contrast, application of GABA\(_A\) receptor antagonist bicuculline (30\(\mu\)M) revealed a robust GABAergic tonic inhibitory current and partially blocked the phasic current. Similarly, GlyRs agonist glycine (500\(\mu\)M) increased the tonic current and decreased the phasic current, while application of GlyRs antagonist strychnine (1\(\mu\)M) only revealed a small glycnergic tonic inhibitory current, and strychnine partially blocked the phasic current. Additionally, co-application of glycine (500\(\mu\)M) and GABA (100\(\mu\)M) following GABA (100\(\mu\)M) application resulted the same changes in tonic current as application of glycine (500\(\mu\)M) alone. Both GABA and glycine hyperpolarized RVLM neurons with a decrease in the input resistances. GlyRs antagonist strychnine had no effects on resting membrane potential of RVLM neurons. In summary, our data revealed that under baseline conditions, GABA\(_A\) receptors determine the basal tone of firing activity in kidney-related RVLM neurons, while GlyRs plays less important role in determining RVLM resting membrane potential. Our data also indicate the presence of unbound extrasynaptic GABA\(_A\) receptors and GlyRs in RVLM neurons, which may be involved in the regulation activity of autonomic nervous system.

This work was supported by NIH COBRE in Hypertension P30 GM-103337.
Polyamine biosynthesis enzymes are critical for the development of the malaria parasite in the mammalian and mosquito hosts. Atif Ghaffar$^{1,2}$, Shaymaa Abdalali$^{1,2}$, Robert J. Hart$^1$, Ahmed S. I. Aly$^1$.

$^1$Department of Tropical Medicine, Tulane University, New Orleans, LA 70112, USA.
$^2$Contributed equally to this work.

Polyamines are important organic charged molecules that play important roles in the cell cycle regulation, cell proliferation, senescence and death of eukaryotes and prokaryotes. In addition, polyamine analogues have been considered and applied in cancer therapy. Despite of the constitutive expression of polyamine biosynthesis enzymes during all malaria parasite life cycle stages, little is known about their biosynthesis and cellular functions for *Plasmodium* development in the mosquito and the mammalian hosts. Herein, we applied gene-targeting techniques in *Plasmodium yoelii* to target enzymes of this pathway for deletion and for fluorescent tagging, with or without the supplementation of polyamines. Our results indicate that polyamines biosynthesis is critical for the development of life cycle stages of *Plasmodium* in the mammalian and the mosquito hosts. Therefore, our data suggest the potential of polyamine biosynthesis enzymes as multi-stage drug targets for antimalarial chemotherapy.
Objective. Experimental stroke in mice with genetic deletion of endothelial nitric oxide synthase (eNOS) showed increased brain infarct size. In contrast, mice with neuronal nitric oxide synthase (nNOS) knockout displayed reduced infarct volume following experimental stroke. Ischemia-reperfusion injury induces oxidative stress that uncouples NOS isoforms leading to production of superoxide instead of nitric oxide. eNOS is constitutively expressed isoform in MECs that primarily produces NO. We hypothesized that MECs constitutively express nNOS isoform that contributes to the generation of superoxide. The objective of the study is to identify the expression of nNOS in MECs and to determine the relative amounts of superoxide produced by nNOS and eNOS.

Methods and Results. Immunohistochemistry identified von Willebrand factor (endothelial marker), eNOS, and nNOS in MECs. The nNOS immunoreactivity to three antibodies raised against different sequences of nNOS was observed in the cytoplasm and also in the nucleus when cells were permeabilized. Superoxide measurements were done by electron spin resonance spectroscopy using selective cell permeable spin trap for superoxide (1-hydroxy-3-methoxy-carbonyl-2,2,5,5-tetramethylpyrroldine; CMH) in the presence of vehicle (DMSO) or selective inhibitors of eNOS (L-N5-(1-Iminoethyl)ornithine; NIO) or nNOS (N-ω-Propyl-L-arginine, NPA). Inhibition of nNOS with 100 nmol/L and 1 μmol/L NPA reduced superoxide generation from MECs to 84±2.5% (n=4, p<0.05) and 72±4.8% (n=10, p<0.05), respectively compared to treatment with vehicle (104±4.4%, n=9). In contrast, inhibition of eNOS with 1 μmol/L of NIO has no significant effect on superoxide generation from MECs (n=10; p=NS).

Conclusions. For the first time, we demonstrated the expression of nNOS in brain microvascular endothelial cells. Unlike eNOS, nNOS appears to produce superoxide constitutively. Thus, nNOS and eNOS likely regulate each other and the function of MECs by altering the balance between the generation of superoxide and nitric oxide mediated signaling pathways.
Background: Accumulation of cardiovascular risk factors and accelerated vascular aging leads to increased vascular stiffness and decreased compliance and elevating pulse pressure (PP). PP, a surrogate marker of arterial stiffness, has been associated with increased risk of cardiovascular mortality, changes in cognitive function and brain structure. Magnetic resonance imaging has demonstrated that variation in PP is related to subtle alterations in white matter structural integrity as assessed by diffusion tensor imaging, and potentially contributing to accelerated brain aging and late life cognitive consequences. Therefore, prolonged exposure to elevations in PP during young adulthood may accelerate cerebromicrovascular disease leading to subclinical changes in the white matter microstructure detectable by DTI.

Methods and Results: Three 25-year trajectories were identified for pulse pressure (PP) in 692 participants (mean age 25 years at baseline), in the CARDIA BRAIN MRI study. The association of trajectory assignment white matter fractional anisotropy by MRI, were investigated. After adjustment for cardiovascular factors (CVF) and anti-hypertension medication use, the most elevated PP trajectories were significantly associated with differences in white matter track integrity, with group mean WMFA being lower when compared to reference.

Conclusions: Our findings suggest that by midlife, prolonged exposure to elevations in mean PP are related to decreases in white matter structurally integrity. Through augmented pulsatility, the microstructural connectivity of the brain is impacted as early as mid-life. As our understanding of the intersection between vascular health and brain health matures, appropriate management of this risk beginning in early adulthood presents a promising modality to decrease morbidity of late life cognitive decline.

This work was supported in part by the NIA/NHLBI Intragency Agreement, the NIA Intramural Program and NHLBI and by the National Institutes of Health (NIH) Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and generous contributions to the Foundation for the NIH from Pfizer Inc, The Leona M. and Harry B. Helmsley Charitable Trust and the Howard Hughes Medical Institute, as well as other private donors. For a complete list, please visit the Foundation website at http://www.fnih.org/work/programs-development/medical-research-scholars-program
INTEGRATION ANALYSIS OF GWAS, HUMAN PROTEIN INTERACTION AND GENE
EXPRESSION IDENTIFIED GENE MODEULES ASSOCIATED WITH BMDs

He H*, Zhang L*,***, Li J*, Wang YP*,**,***, Zhang JG*, Deng HW*,**

*Center for Bioinformatics and Genomics, Department of Biostatistics and Bioinformatics,
Tulane University, New Orleans, LA, 70112, USA
**Biomedical Engineering Department, Tulane University, New Orleans, LA, 70118, USA
***Center of System Biomedical Sciences, University of Shanghai for Science and Technology,
Shanghai 200093, P. R. China

To date, few systems genetics studies in bone field have been performed. We designed our
study from a systems-level perspective by integrating GWASs, human protein-protein
interaction (PPI) network and gene expression to identify gene modules contributing to
osteoporosis risk.

First we searched for modules significantly enriched with BMD-associated genes in human PPI
network by using two large meta-analysis GWAS datasets through a dense module search
algorithm. One was an imputation-based meta-analysis (referred to as Meta7), including seven
GWAS samples. The other was the second GWAS meta-analysis from Genetic Factors for
Osteoporosis (GEFOS) Consortium (GEFOS-2). One was assigned as a discovery dataset and
the other as an evaluation dataset, and vice versa. Through such a discovery-evaluation
strategy, in total 42 modules and 129 modules were identified significantly in both Meta7 and
GEFOS2 dataset, for FN and SPN BMD respectively. And there were 3340 modules identified
for Hip BMD only in Meta7. Next, as candidate modules, they were assessed the biological
relevance to BMD by gene set enrichment analysis (GSEA) in two independent microarray
expression datasets. Both datasets consisted of expression profiles generated from circulating
monocytes isolated and purified in subjects with low versus high BMD values. Interestingly,
there were two modules significantly enriched in monocytes from low BMD group in both gene
expression datasets (nominal p value < 0.05). Two modules overlapped in their gene content
and had 16 nonredundant genes, including ESR1, LRP5, TNFRSF11B, SMAD3, SFRP1,
SFRP2, MDF1, LRP5, WNT1, WNT4 and DKK1, etc. Functional enrichment analysis further
revealed that both modules were enriched for genes involved in Wnt receptor signaling and
osteoblast differentiation.
From a system-level analysis above, we highlighted two modules as well as genes in modules playing important roles in the regulation of bone mass, providing important clues for therapeutic approaches for osteoporosis.

This work was partially supported by grants from the National Institutes of Health [P50AR055081, R01AG026564, R01AR050496, RC2DE020756, R01AR057049, and R03TW008221]; and startup funds from Tulane University, University of Missouri – Kansas City.
DIRECT DELIVERY OF POLAR CARGO TO LIPID VESICLES AND CELLS BY SPONTANEOUS MEMBRANE-TRANSLOCATING PEPTIDES

He J*, Fuselier T*, Kauffman WB*, Naveen Sk**, Voss TG**, Hristova K*** and Wimley WC*

* Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA 70112

** Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112

*** Department of Materials Science and Engineering, The Johns Hopkins University, Baltimore, MD 21218

The hydrophobic barrier of the membrane prevents simple cellular uptake of many useful exogenous polar compounds in the laboratory and clinic. For the past few decades, cell-penetrating peptides (CPPs) have been intensively studied as a potential cargo delivery vehicle. However, it has gradually been accepted that the actions of CPPs rely on one or multiple types of endocytic pathways, which can make cargo delivery quite inefficient under some conditions. Therefore, this work presented here was initiated to engineer spontaneous membrane-translocating peptides (SMTPs) that move across lipid bilayers and cellular membranes in an endocytosis-independent manner. Previously we used high throughput, orthogonal screen of a synthetic peptide library and identified a family of 12-residue SMTPs that translocate rapidly without causing any bilayer destabilization. Here we conjugated one of the SMTPs described above with several membrane-impermeant molecules and measured the rate of translocation in large unilamellar vesicles. Furthermore, we characterized the cytosolic entry of SMTP-polar cargo conjugates as well as their toxicity in living mammalian cells. We observed rapid delivery of these conjugates with higher efficiency compared to control CPP-cargo conjugates, and little or no evidence for the involvement of endocytosis in SMTP translocation. Meanwhile, we found no measureable cytolytic activity and extremely low cytotoxicity of SMTPs. Upon injection into mice, SMTPs with their cargos were found in many tissues after 2 hours, while free cargo molecules were rapidly cleared and not systemically distributed. Our results show that SMTPs behave in a unique way compared to conventional CPPs: they may strategically elude the dependence of endocytosis in cellular translocation and significantly improve cytosolic delivery of polar cargos.
DUAL ROLE OF MEK1/2 AND MEK5 IN THE REVERSAL OF EPITHELIAL-TO-MESenchymal TRANSITION


Department of Medicine, School of Medicine*, Biomedical Engineering Department****, Tulane University, New Orleans, LA

Division of Medicinal Chemistry, School of Pharmacy**, Division of Pharmacology, School of Pharmacy***, Duquesne University, Pittsburgh, PA

The mitogen-activated protein kinase (MAPK) pathway has well-established roles in cellular processes including proliferation, differentiation, and regulation of cell fate, namely survival and apoptosis. In breast cancer, constitutive activation of the MAPK/extracellular signal-regulated kinases (ERK) pathways have been linked to chemoresistance and metastatic progression through distinct mechanisms including the activation of epithelial-to-mesenchymal transition (EMT). Our previous studies have shown that overexpression of MEK5 promotes EMT markers and induces the progression to a mesenchymal phenotype. Here, we tested the effects of a novel MEK1/2 and MEK5 inhibitor, SC-1-151, and other known MAPK signaling inhibitors (PD184,352 (MEK1/2), AZD6244 (MEK1/2), BIRB796 (p38)) on a panel of mesenchymal and highly metastatic breast cancer cell lines. While the MEK1/2 and p38 inhibitors decreased cell viability across cell lines, only the dual inhibition of MEK1/2 and MEK5 though the use of SC-1-151 demonstrated a change in cell morphology indicative of mesenchymal-to-epithelial transition (MET). Furthermore, the cells exhibited a significant decrease in migration potential following SC-1-151 treatment.

Further analysis of the effects of SC-1-151 in the triple-negative breast cancer cell lines revealed an alteration of the genes associated with EMT, notably a decrease in expression of Fra-1, a transcription factor downstream of MAPK. Immuno-compromised mice inoculated with the MDA-MD-231 cell line and treated with SC-1-151 demonstrated decreased tumor volumes compared to vehicle-treated animals at day 30 post cell injection, implicating the role of MEK inhibition on tumorigenesis. These data demonstrate the need for a better understanding of the dual role of MEK1/2 and MEK5 signaling in breast cancer, and suggest that inhibition of the MEK1/2 and MEK5 signaling pathways leads to a decrease in EMT and cell migration.
LOW-CARBOHYDRATE DIETS AND INCIDENCE, PREVALENCE, AND PROGRESSION OF CORONARY ARTERY CALCIUM IN THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS (MESA)

Hu T*, Jacobs DR**, Nettleton JA***, Steffen L**, Bertoni A****, He J*, Bazzano LA*

* Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA
** Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN
*** The University of Texas Health Science Center, Houston, TX
**** Wake Forest School of Medicine, Winston-Salem, NC

Background: The coronary artery calcium (CAC) score is associated with the risk of coronary heart disease. We aimed to assess the relationship between low-carbohydrate dietary patterns and CAC scores in the MESA cohort.

Methods: Our sample included 5,702 men and women who were free of clinical cardiovascular disease and had food frequency questionnaires at baseline (2000-2002), and at least one measure of CAC during follow-up. We excluded those with implausible energy intake (<600 kcal/day or >6000 kcal/day) or daily physical activity (>24 hours). Two low-carbohydrate-diet (LCD) scores were generated: an overall LCD score was calculated based on total carbohydrate, fat, and protein, and a plant-based LCD score was calculated using intakes of unsaturated fat (excluding trans fat) and vegetable protein. CAC scores at exam 1 and at 2 and 3 (18 and 36 months later) were used in multivariable relative risk regression models to examine the association between LCD scores and CAC prevalence and incidence (binary), while robust regression was used to examine CAC progression (continuous). Analyses were adjusted for demographic, socioeconomic, lifestyle, and cardiovascular risk factors.

Results: The mean age was 62 years, 48% of participants were male, and 40.8% were White. The mean (SD) levels of carbohydrate intake as a percentage of energy were 64.2 (5.2), 56.1 (4.9), 51.5 (3.7), 47.5 (4.0), and 42.1 (5.6) from the lowest to the highest quintiles of the overall LCD score. There were 2,652 (46.5%) participants who had positive CAC scores at baseline and 252 participants who had newly positive scores for CAC during follow-up. Among those with prevalent CAC at baseline, the median (IQR) of increases in CAC was 47 (132) over follow-ups. For incident CAC, relative risk estimates (95% CI) from Quintile 1 to 5 were 1, 0.73 (0.52, 1.02), 0.65 (0.45, 0.95), 0.90 (0.63, 1.28), 1.05 (0.77, 1.42) for overall LCD scores, and were 1, 1.14 (0.81, 1.61), 0.98 (0.71, 1.37), 1.08 (0.78, 1.49), 1.15 (0.82, 1.62) for plant-based LCD scores, respectively. No significant trend was observed for associations with incident CAC. There was no significant association between any LCD score and CAC prevalence or progression among those with positive CAC scores at baseline.

Conclusions: A low-carbohydrate diet, including a plant-based low-carbohydrate diet, was not associated with prevalence, incidence, or progression of CAC among those with prevalent CAC at baseline.

Grant Acknowledgement: This research was supported by contracts N01-HC-95159 through N01-HC-95166 and N01-HC-95169 from the National Heart, Lung, and Blood Institute (NHLBI) for the collection of data in the MESA study. Dr. Bazzano was supported by R01 AG041200
from the NIA. NIA is an component of the National Institutes of Health (NIH). The authors would like to thank other investigators, the staff, and the participants of the MESA study for their valuable contribution.
Mutant ZP1 in Familial Infertility

Hua-Lin Huang, M. D., Chao Lv, M.D., Ying-Chun Zhao, Ph.D., Wen Li, Ph.D., Xue-Mei He M.D., Ping Li, M.D., Ai-Guo Sha, M.D., Xiao Tian, Christopher J. Papasian, Ph.D., Hong-Wen Deng, Ph.D., Guang-Xiu Lu, M.D., and Hong-Mei Xiao M.D., Ph.D.

SUMMARY

The human zona pellucida is composed of four glycoproteins (ZP1-ZP4) and has an important role in reproduction. Here we describe a form of infertility with an autosomal recessive mode of inheritance, characterized by abnormal eggs that lack a zona pellucida. We identified a homozygous frameshift mutation in \textit{ZP1} in six members of the family. \textit{In vitro} studies showed that defective ZP1 protein and normal ZP3 co-localized throughout the cells and were not expressed at the cell surface, suggesting that the aberrant ZP1 results in the sequestration of ZP3 in the cytoplasm, thereby preventing the formation of the zona pellucida around the oocyte.
CHARACTERIZATION OF EMBRYOID BODIES SEPARATED USING DENSITY GRADIENT CENTRIFUGATION

James DE*, Ahsan T*
*Department of Biomedical Engineering, Tulane University, New Orleans, LA

Embryonic stem cells (ESCs) are pluripotent and spontaneously differentiate into all cell types. They are highly proliferative and capable of being cultured in basic medium and grown in suspension as embryoid bodies (EBs). EBs recapitulate early development, however, with longer culture times they become more heterogeneous. We posit that as EBs become more heterogeneous this will likely be reflected in their density as a result of variations in cavitation, extracellular matrix deposition and cellular organization. This variation in EB density should allow embryoid bodies to be separated via density gradient centrifugation using Percoll (a solution commonly used for the separation of blood cells). D3 murine embryonic stem cells were expanded and placed in culture in rotary suspension to form EBs. EBs at various time points were subjected to density gradient centrifugation. The ‘switch-point’, or point at which the EBs switch between being more to less dense than the Percoll was in the 1.035-1.055 g/ml range. We then created stacked gradients of Percoll within this range at intervals of 0.005 g/ml. After centrifugation EBs settled at the interface between the layers of Percoll. These EBs were collected and subjected to morphologic and RNA expression analysis. It was found that more dense EBs are smaller in area and more homogeneous in size than the least dense fraction. It was also discovered with qRT-PCR analysis that markers for pluripotency such as Nanog, OCT4 and SOX2 were all upregulated compared in the denser fractions. AFP, a marker of endoderm was upregulated in less dense fractions. Our work has shown that it is possible to isolate EBs into distinct subpopulations with density gradient centrifugation. We have also shown that these subpopulations differ in expression of pluripotency and endodermal markers. It is possible to purify a more enriched population of target tissues or purify out potentially tumorigenic pluripotent populations. This method of enriching EBs can be upscaled to yield large amounts of desired phenotypes.
SIGNALING PATHWAYS ACTIVATED BY SHEAR STRESS DURING EMBRYONIC STEM CELL DIFFERENTIATION

Janaszak MM*, Wolfe RP*, Ahsan T*

*Department of Biomedical Engineering, Tulane University, New Orleans, LA

Embryonic stem cells (ESCs) have the ability to give rise to any somatic phenotype, and therefore have the potential to be used in tissue engineering applications and cell therapies. Shear stress has been shown to promote mesodermal differentiation, however, the cellular pathways and signaling mechanisms involved in shear mediated differentiation are poorly understood. Studies of the signaling cues involved in these pathways can allow for improved control over cell specification, and enhanced scale-up differentiation techniques. The objective of this study was to determine if specific signaling pathways are involved in the shear mediated differentiation of ESCs by using small molecule inhibitors.

ESCs were seeded onto collagen type-IV-coated slides and cultured for two days in static conditions to allow for cell attachment. Thereafter, two days of either continued static treatment or shear stress treatment ($\tau=1.5 \text{ dyn/cm}^2$) was applied to the cells. Culture medium was supplemented with small molecule inhibitors for either c-SRC, ROCK, JNK, or ERK. RT-PCR was used to evaluate the gene expression levels of FLK1, TIE2, and RUNX1 as markers of mesodermal, endothelial, and hematopoietic differentiation, respectively. Statistical differences in gene expression were evaluated using paired t-tests.

Cells treated with 2.5µM c-SRC inhibitor SU6656 were shown to block regulation of FLK1 by shear stress, but not TIE2 and RUNX1. No significant effect on shear mediated upregulation of FLK1, TIE2, or RUNX1 was seen for cells treated with 10mM ROCK inhibitor Y27632. Inhibition of JNK with 10µM SP600125 showed that shear modulation of FLK1 and RUNX1 are dependent on the JNK pathway. Lastly, cells treated with 10µM ERK inhibitor U0126 showed ERK may modulate shear mediated regulation of TIE2 and RUNX1, but not FLK1. Overall, these studies show that the response to shear stress was affected by c-SRC, JNK, and ERK, but not ROCK. Furthermore, they show that inhibition of ERK diminished differentiation towards endothelial and hematopoietic phenotypes, meaning that this pathway may be involved in the response to shear stress.

Follow up of these preliminary studies will elucidate the poorly understood cellular mechanisms and signaling pathways by which environmental cues, such as shear stress, affect differentiation towards specific phenotypes. This type of mechanistic understanding will ultimately help with the rationale design of directed differentiation approaches to generate vascular phenotypes.
WHOLE GENOME EXPRESSION PROFILING OF RIFT VALLEY FEVER VIRUS (RVFV) INFECTION: A COMPARISON OF AVIRULENT MP12 VERSUS WILD-TYPE ZH501

John* K, Hill** T, Freiberg*** A and Panganiban* A
*Division of Microbiology, Tulane National Primate Research Center, Covington, LA; **Department of Pathology, University of Texas Medical Branch, Galveston, TX

RVFV is a zoonotic arbovirus that can devastate herds of domestic ruminants, and cause disease in humans. Though predominantly endemic to parts of Africa, its relatively recent spread to Egypt and certain parts of the Arab world have become a cause for concern. Given its potential for rapid spread and causation of morbidity and/or mortality it has listed as a ‘select agent’ by the CDC and a ‘Category A’ agent by NIAID. We used whole genome expression analysis to better define the molecular signatures of RVFV infection. The goal was to identify novel gene targets with potential roles in virus infectivity, replication and pathophysiology. Two strains of RVFV, MP12 (an attenuated ‘vaccine’ strain of the virus) and ZH501 (wild-type virus) were used. Gene expression changes in RVFV-infected human primary vascular endothelial cells were monitored 24, 48, and 72 hours post infection (PI). Genes significantly (p<0.05) up (fold change ≥ 2.0) or down-regulated (fold change ≤ 2.0) were considered altered. As expected, ZH501 altered the expression of a greater number of genes relative to MP12 (334 versus 139, respectively). However, early responses, 24 h PI, were more pronounced in MP12 than ZH501 (93 genes altered by MP12 versus 49 by ZH501). Maximal changes in host gene expression took place at 48 h PI in both MP12 and ZH501 infected cells. A set of 28 genes with likely roles in various processes of RVFV infection were chosen for further qPCR validation. Three genes, CCL5, TLR3 and MGP correlated well with expression patterns on microarrays and also exhibited a significant difference in expression between MP12 and ZH501 at 48 h, 72 h and 72 h, respectively. Expression of four genes was not detectable. Further studies are underway to validate some of the identified targets through functional and proteomic approaches. In addition we are examining correlation of these genes with specific of disease and cellular signaling pathways that may be affected by RVFV infection.

For presentation at the Health Sciences Research Days, April-2-3, 2014, Tulane University, New Orleans, LA
Orthopaedic surgeons performed more than 500,000 total knee arthroplasties (TKA) in the past year, making it one of the most common surgical procedures in the US. Estimates project a dramatic increase to 3.48 million cases per year by 2030. As such, it is important to identify and minimize risk factors that predict a negative outcome. A retrospective chart review was undertaken to compare the incidence of postoperative wound complications in patients undergoing TKA treated with either an incisional wound vac—also known as negative pressure wound therapy (NPWT)—or traditional soft dressing, composed of Xeroform®, sterile gauze, abdominal pads, and tape. 103 patients were evaluated over a 6-month period following TKA, 60 (40 primary and 20 revision) with NPWT and 43 (24 primary and 19 revision) with traditional soft dressing. Incidence of wound complications were 23% and 28% in the NPWT and soft dressing groups, respectively. In examining the severity of the complication, 7% of those receiving a standard dressing required revision surgery compared to 3% in the NPWT group. Our study demonstrates that postoperative NPWT is associated with decreased severity of postoperative wound complications as well as wound complication rate. These findings support that NPWT is superior to traditional soft tissue dressing in a postoperative setting for TKA.
Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) cause massive and progressive depletion of CD4+ T cells in both peripheral and mucosal tissues. Cellular immune responses are temporarily associated with the control of plasma viremia in the acute stage of HIV/SIV infection. However, virus-specific T-cell responses are not efficient enough to completely control viral replication. Therefore, a better understanding of the immune signaling pathways of different T-cell subsets is important to understand the early key defects in T-cell mediated immune responses in HIV/SIV pathogenesis. The aim of this study was to understand the transcriptional dynamics of CD4+ and CD8+ T cells following SIV infection. Three rhesus macaques (RMs) were infected with 300-500 TCID50 SIVMAC251 intravaginally and peripheral blood was collected at various intervals after SIV challenge, to determine CD4/CD8 dynamics, proliferation (Ki67) and the stage of differentiation (CD7). All SIV-infected RMs possessed significantly higher plasma viral load with a significant loss of peripheral CD4+ T cells, which exhibited increased proliferation and differentiation markers, as opposed to only enhanced proliferation in CD8+ T cells. We also carried out whole genome expression analyses (Rhesus Macaque Genome Array, Affymetrix) in purified CD4+ and CD8+ T-cell populations, isolated from peripheral blood of uninfected and SIV-infected RMs at 16, 26 and 49 days post SIV infection (dpi). Expression profiles from purified T-cell subpopulations isolated from three RMs were compared to one uninfected control. Whole genome expression analyses revealed significant changes in gene expression (fold change ≥2.0 infected vs. control, p<0.05) in both CD8+ (11341 probe-sets) and CD4+ T-cell subsets (2601 probe-sets). The majority of the gene expression changes were manifested earlier in CD4+ T cells (2455 probe-sets altered at 16 dpi) relative to CD8+ T cells (11301 probe-sets altered at 26 dpi). Ingenuity Pathway analysis associated these genes with gastrointestinal disease, cell-cell signaling and inflammatory response pathways in CD4+ T cells. Interestingly, at 26 dpi the majority of significantly altered genes were downregulated, including interleukin 8 (IL-8) and pro-platelet basic protein (PPBP) which both produce immunoregulatory chemokines, indicative of dysfunction in CD4+ T cell population. Conversely, at 49 dpi there were no significant pathway alterations in this cellular subtype. In CD8+ T cells, the significantly modified pathways included antimicrobial responses, inflammatory responses, cancer and gastrointestinal diseases. As with CD4+ T cells, there was downregulation of IL-8 and genes encoding other important immunoregulatory cytokines such as tumor necrosis factor (TNF) at 26 dpi. However, unlike CD4+ T cells, there was also an upregulation of genes encoding important cytokines and chemokines, such as chemokine (C-C motif) ligand 5 (CCL5), which has a proposed role in viral control. Further studies are still underway to validate some of altered targets and delineate possible underlying mechanisms of T-cell dysfunction during SIV pathogenesis.

Work Supported by NIH/NIAID- R21 AI080395

For presentation at the Tulane Health Sciences Research Day, April 2-3, 2014, Tulane University, New Orleans, LA
Prostate cancer (PCa) is the second leading cause of cancer-related mortality in American men. While androgen ablation therapy (AAT) is effective in treating primary tumors, a large number of patients regress to a more aggressive phenotype known as Castration Resistant Prostate Cancer (CRPC). Currently, a chemotherapy regimen is the only approved indication against CRPCs; however, chemotherapy-associated toxicities significantly increase patient morbidity. Therefore, it is necessary to design a novel therapeutic approach to enhance the efficacy of currently available AAT regimen, either before or after the initiation of therapy. Towards this goal, the utility of drugs that are already approved for other indications, i.e. a drug repositioning approach, will be of significant clinical benefit. Nelfinavir (NFR), a FDA-approved HIV protease inhibitor, is known to suppress tumor growth via the inhibition of PI3K/AKT survival pathway and the induction of Autophagy. Furthermore, the phytochemical Curcumin (CUR) has demonstrated anti-proliferative effects on cancer cells by inhibiting both NF-κB and PI3K/AKT pathways. Since these crucial metabolic pathways are linked to aggressive tumor growth and hormone resistance, we hypothesized that physiologic concentrations of NFR and CUR may inhibit CRPC growth and sensitize them to AAT. Preliminary findings in C4-2B cells (a CRPC line) showed a concentration dependent suppressive effect of both NFR and CUR, under normal growth conditions (10% FBS). Interestingly, under hormone depleted conditions (10% CS-FBS), NFR increased CRPC growth but CUR suppressed it. Furthermore, pre-exposure to NFR (5 μM) and subsequent coexposure to CUR (10 μM) was optimum in inhibiting growth of C4-2B cells growing in 10% FBS. However, this combination therapy failed to inhibit C4-2B growth under 10% CS-FBS condition. These findings suggest that Autophagy, AKT and NF-κB signaling pathways are important regulators of growth and hormone signaling in CRPC cells, and further molecular studies using these agents may provide a promising therapeutic strategy to suppress growth of prostate tumors. Our findings may offer a novel and safe therapeutic alternative to toxic chemotherapy to treat the aggressive CRPC cells.
PATHOPHYSIOLOGY OF CONTRAST-INDUCED NEPHROPATHY IN ELDERLY DIABETIC MICE


*Section of Nephrology & Hypertension, **Peptide Research Laboratory, Department of Medicine, Tulane University, School of Medicine, New Orleans, LA; ***Department of Physiology, School of Medicine, LSUHSC, New Orleans, LA; and ****Veterans Affairs, Southeast Louisiana Veterans Health Care System, New Orleans, LA.

Purpose: Contrast-induced nephropathy (CIN) is associated with significant clinical and economic consequences, including prolonged hospitalization and dialysis. CIN can lead to renal failure and death, especially in elderly patients with diabetes. CIN is primarily the result of medullary hypoxia, oxidative stress and the direct toxic effects of contrast media (CM) on renal epithelial and endothelial cells. A novel CIN model was developed in aged homozygous db/db mice which spontaneously develop diabetes and nephropathy. This novel preclinical CIN model more closely resembles the patients that are at high risk of developing CIN. We hypothesized that db/db mice would be more vulnerable to CIN than non-diabetic mice.

Methods: We studied 12-wk and 24-wk-old (n = 3-5) diabetic db/db and non-diabetic wild-type (WT, C57Bl/6) male mice. Mice were dehydrated for 24 hr and nonionic low osmolar (iohexol) or isosmolar (iodixanol) CM was injected via a catheter through the jugular vein at a dose of 3 g of iodine/kg b.w. All mice were sacrificed at 24 or 72 hr after CM injection. Sham-operated mice were injected with the same volume of saline as CM. After 24 hr or 72 hr injection, renal function was assessed by directly measuring glomerular filtration rate (GFR) using a single bolus inulin-FITC method and renal injury was quantified by measuring serum creatinine, kidney injury biomarkers, proteinuria, and histopathology.

Results: At 12 wk of age, diabetic and control mice did not show signs of CIN. Older db/db mice (24 wk) showed a significant increase in their body weight, 24 hr urine excretion and kidney weights compared to WT mice (p < 0.001). These elderly db/db mice were diabetic as shown by a significant increase in 24 hr urinary glucose levels (p < 0.001), proteinuria, and ketonuria, and a significant decrease in pH (p < 0.05) in 24-hr urine samples compared to WT mice. After 24 hr of CM injection, diabetic mice had a significant decrease in GFR (iodixanol = 337 ± 9.94 µl/ml, p < 0.01; iohexol = 209 ± 0.0, p < 0.001) compared to sham-operated db/db mice (413 ± 3.59 µl/mim), a significant increase in kidney injury as measured by serum creatinine (iohexol = 55 ± 5 µg/dl, p < 0.05) compared to sham-operated db/db mice (10 ± 0.0 µg/dl), and levels of kidney injury molecule-1 (KIM-1) in urine (both CM, p < 0.5) compared to sham-operated db/db mice. Both CM caused significant structural damage to kidney cells in 24-wk old db/db mice at 24 hr post CM injection compared to sham-operated db/db mice.

Conclusions: At an age of ≥ 24 wk, db/db mice have increased diabetic nephropathy and they are more prone to CIN than younger mice. Iohexol is more nephrotoxic than iodixanol. This diabetic mouse model could be used for the development of novel therapeutic strategies to prevent CIN.

This work was supported by a PTRF research grant from DCI.
Methicillin-resistant *Staphylococcus aureus* (MRSA) infections remain a major public health concern in the United States and other developed countries, claiming responsibility for 5,500 U.S. deaths in 2007 and resulting in annual U.S. economic burden estimates up to $13.6 billion. Of particular concern is the increasing prevalence of highly virulent and transmissible community-associated MRSA (CA-MRSA) infections in healthy individuals outside of the hospital setting. Due to the emerging antibiotic resistance of the associated strains, clinicians and scientists are searching for new approaches to combat MRSA, including the development of anti-virulence therapies. A majority of *S. aureus* virulence factors are directly under the control of the *agr* regulator, including the regulation of surface binding proteins, exoenzymes, and nearly all *S. aureus* toxins. Additionally, the *agr* operon is responsible for *S. aureus* quorum-sensing, a process that contributes to MRSA’s opportunistic ability to evade the host immune response and establish infection. This review examines the potential to target *agr*-regulated quorum-sensing for novel anti-virulence drug development against MRSA.
Enrichment of T6SS-1 proteins in an outer membrane vesicle vaccine against *Burkholderia pseudomallei*

Kikendall N, Petersen H, Morici L
Microbiology and Immunology, Tulane University School of Medicine, New Orleans LA

Abstract:
*Burkholderia pseudomallei* (*Bp*) is the causative agent of melioidosis, a disease with septicemic and pneumonic clinical manifestations which causes high morbidity and mortality in endemic regions. *Bp* is listed as a Tier 1 select agent by the US DHHS and is considered a potential biological threat. *Bp*, an intracellular pathogen, is inherently resistant to multiple antibiotics, requires intensive treatment and causes relapse in more than 25% of survivors ([Limmathurotsakul:2011ko]). Traditional vaccine strategies have proven inadequate against *Bp* and immune responses remain largely uncharacterized. Our lab has previously demonstrated that parenteral immunization with naturally-derived *Bp* outer membrane vesicles (OMVs) provides significant, although incomplete, protection against lethal sepsis and pneumonic melioidosis in mice ([Nieves:2011hm]). Due to the complex pathogenicity of *Bp*, a completely effective vaccine will likely require induction of both antibodies and T cell responses to essential virulence determinants. We hypothesize that bacterial surface proteins specifically upregulated during intracellular infection are essential targets for establishing sterilizing immunity. We postulate that incorporation of these proteins into the multivalent OMV vaccine platform will elicit the necessary range of immune responses, including antibody, helper CD4+ and cytotoxic CD8+ T cells. Expression of bacterial surface proteins belonging to the Type 6 Secretion System (T6SS-1) is precisely regulated during the intracellular stage of infection and is critical for bacterial survival within the host cell. Culture mediums, such as minimal media with casamino acids (M9CG), that mimic the macrophage intracellular environment promote the upregulation of T6SS proteins in broth-grown *Bp* ([Burtnick:2013ig]). In this study, we show that OMVs purified from cultures of *Bp* strain 1026b grown in M9CG express Hcp-1, a component of T6SS-1. OMVs were confirmed visually by transmission electron microscopy and a Western blot was performed to confirm the presence of Hcp-1. LC/MS analysis provided a comprehensive evaluation of the protein composition of OMVs derived from *Bp* grown in M9CG compared to OMVs derived from *Bp* grown in Luria Broth media. These results confirmed the expression of essential T6SS-1 proteins in OMVs derived from M9CG grown *Bp*. OMVs were used to immunize BALB/c mice (n=10) at a concentration of 2.5 µg OMV with alum (adjuvant) or alum only on days 0, 21, and 42. Retro orbital bleeds were performed prior to each immunization, and serum was collected for antibody analysis. Mice were challenged by whole body aerosol with 2x10^6 CFU of *Bp*1026b on Day 70 to determine whether incorporation of specific antigens in the OMV vaccine promoted protection. Mice immunized with the enhanced OMVs were significantly more protected than those immunized with adjuvant only. In future studies, we will identify and incorporate additional surface proteins necessary for *Bp* intracellular survival into the current OMV vaccine to further enhance protection.
Prostate cancer (PCa) is the most common and the second leading cause of cancer-related death among males in the Western world. TMPRSS2, a gene that encodes serine-protease type II transmembrane domain, has been shown to be frequently prevalent as a TMPRSS2-ERG gene fusion during early event in the genesis of PCa. However, TMPRESS2 function remains largely unknown, especially in castration-resistant PCA cells. While TMPRESS2 has previously been shown to be an androgen-regulated gene, TMPRESS2–ERG expression is regulated by a novel ERβ-dependent mechanism. The objectives of this study are to determine the mechanisms of expression and the functional significance of TMPRSS2 in growth and migration of castration-resistant AR-null PC-3 cells. 17β-Estradiol (E2; 10nM) induced TMPRSS2 expression (4-fold higher) in an ERβ-dependent manner as evidenced by shRNA silencing, but not by ERα-specific shRNA, in PC-3 cells. Intriguingly, the E2-induced TMPRSS2 expression was blocked by AG1024 (IGF-1R inhibitor) or knockdown of IGF-1R by siRNA, but not by AG1478 (EGFR inhibitor), suggesting that IGF-1R mediates expression of TMPRESS2 by the E2-ERβ axis in PC-3 cells. Moreover, TMPRSS2-specific siRNA markedly attenuated E2-induced cell growth and migration of PC-3 cells, suggesting that TMPRSS2 may be pivotal to E2-mediated progression of castration-resistant disease in vivo. The effects of TMPRSS2 silencing seem to be mediated through inhibition of E2-induced activation of NF-κB-signaling in these cells. Taken together, our results unraveled the underlying mechanisms that govern the expression and function of TMPRSS2 in mediating E2-induced growth and migration of castration-resistant PC-3 cells. Delineation of such critical players may culminate in identifying therapeutic targets to selectively circumvent castration-resistant outgrowth in PCa patients.

Keywords: Prostate cancer PC-3, TMPRESS2, ERβ, IGF-1R, NF-κB
Expression of the Long INterspersed Element-1 (LINE-1 or L1) retrotransposon, the only autonomous family of mobile elements currently active in the human genome, can disrupt genomic integrity through insertional mutagenesis, deletions generated through non-allelic homologous recombination, and the creation of DNA double-strand breaks (DSBs). Additionally, L1 contributes to further insertional mutagenesis through the mobilization of other transposable elements such as Alu and SVA, which rely on the L1 ORF2 protein for their propagation. These types of L1-induced mutagenic events have contributed to genomic instability in a variety of genetic diseases and cancers.

The endonuclease encoded from the L1 ORF2 protein initiates the retrotransposition process by nicking the first strand of the DNA. It has been recently reported that expression of the L1 endonuclease generates DSBs at a rate 10- to 100-fold higher than the rate of L1 insertion events, suggesting that the genomic damage caused by L1-induced DSBs could be significantly greater than the damage resulting from L1 insertions. The vast majority of the roughly 500,000 L1 loci in the human genome are no longer capable of retrotransposition due to 5'-truncations or the acquisition of premature stop codons or other deleterious mutations. However, some retrotranspositionally-incompetent L1 (ri-L1) loci are still expressed and may be capable of causing genomic instability through means other than retrotransposition. Expression of truncated ORF2 proteins from ri-L1 loci containing premature stop codons could potentially have a significant impact on genomic integrity if the endonuclease domain remains functional.

In this study, we investigated the potential for ri-L1 to produce truncated ORF2 proteins, and characterized the subsequent cellular toxicity and genomic damage generated by their expression. Results from western blot analysis and toxicity assays demonstrate evidence of cellular toxicity and DNA damage due to the expression of some truncated L1 ORF2 proteins. Transient expression of some ORF2 constructs containing premature stop codons were also able to cause cellular toxicity and genomic damage in mammalian cells, and furthermore led to Alu retrotransposition. Our data provide experimental evidence that a proportion of L1 loci in the human genome, previously considered to be harmless to genomic integrity, may be capable of inducing some level of genomic instability through the generation of DSBs and Alu mobilization.
OBESITY-RELATED GENOMIC LOCI ARE ASSOCIATED WITH TYPE 2 DIABETES IN A HAN CHINESE POPULATION

Kong XM*, Zhao Q*, He J*, Yang WY**

*Department of Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, LA, US
**Department of Endocrinology, China-Japan Friendship Hospital, Beijing, China

Obesity is a well-known risk factor for type 2 diabetes (T2D), presumably through its effect on insulin resistance. Recent genome-wide association studies have identified many genomic loci associated with obesity and its related traits. However, associations between obesity-related genomic loci and T2D are unclear. The aim of this study is to examine the associations between established obesity-related genomic loci and T2D in a Han Chinese population. We genotyped 18 obesity-related single nucleotide polymorphisms (SNPs) (within or close to NEGR1, SEC16B, TMEM18, ETV5/DGKG, BAT2, BDNFOS, BDNF, FAIM2, MC4R, KCTD15, MTCH2, GNPDA2, NPC1, MAF, PRL, MSRA, SLC30A10 and TFAP2B genes) among 5,338 T2D cases and 4,663 controls from the Chinese National Diabetes and Metabolic Disorders Study. The associations of these SNPs with T2D and quantitative glycemic traits (in controls) were analyzed using logistic and linear regression models, respectively. An additive genetic model was assumed. Two SNPs near MC4R (rs12970134) and GNPDA2 (rs10938397) genes were significantly associated with T2D after adjusting for age and sex (OR [95% CI] = 1.14 [1.06, 1.22] for the A allele of rs12970134, \( P = 4.75 \times 10^{-4} \); OR [95% CI] = 1.10 [1.03, 1.17] for the G allele of rs10938397, \( P = 4.54 \times 10^{-3} \)). When body mass index (BMI) and waist circumference (WC) were further adjusted, the association between GNPDA2 and T2D was attenuated (OR [95% CI] = 1.06 [0.98, 1.14], \( P = 1.26 \times 10^{-1} \)). However, the association of MC4R with T2D remained significant (OR [95% CI] = 1.10 [1.02, 1.20], \( P = 1.81 \times 10^{-2} \)) after adjusting for BMI and WC. Joint effect analyses showed that a genetic risk score, calculated from the sum of the obesity risk alleles for each individual, was significantly associated with increased risk for T2D, even after adjusting for BMI and WC. Compared to the lowest quartile of the genetic risk score, the ORs [95% CI]) were 1.05 [0.85, 1.17], 1.18 [1.03, 1.34], and 1.37 [1.19, 1.58] for the other three quartiles after adjusting for BMI and WC (\( P \) for trend=0.0001). In addition, five SNPs (near or within SEC16B, BDNF, MTCH2, MAF and PRL genes) showed significant associations with quantitative glycemic traits, such as glucose and insulin levels during the oral glucose tolerance test, in controls both before and after adjusting for BMI and WC (all \( P \) values < 0.05). In conclusion, this study indicates that obesity-related genomic loci may contribute to the risk of T2D and explain the variations of quantitative glycemic traits in the Han Chinese population. They may also have independent effects on T2D beyond BMI and WC. Furthermore, the present study may enhance the current understanding of the role of obesity in the pathogenesis of T2D.

The Chinese National Diabetes and Metabolic Disorders Study is supported by grants from the Chinese Medical Association Foundation and Chinese Diabetes Society. Dr. Qi Zhao is supported by Award K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institute of Health.
RECOMBINANT PFS25 PRODUCED IN *E. coli* INDUCES HIGHLY POTENT *PLASMODIUM FALCIPARUM* TRANSMISSION BLOCKING IMMUNITY

Kumar Rajesh*, Angov Evelina**, Kumar Nirbhay*

Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street (SL-17), New Orleans, LA 70112*, Walter Reed Army Institute for Research, Silver Spring, MD 20910 **.

Pfs25 expressed on the surface of zygotes and ookinetes, is a well-established malaria transmission blocking vaccine target antigen. Production of Pfs25 in native functional conformation with potential to elicit effective immunogenicity without need for any further chemical conjugation still remains a major challenge. In the current study, codon harmonized recombinant Pfs25 (CH-rPfs25) was expressed in *E. coli* in monomeric form retaining reduction-sensitive conformational epitopes of transmission blocking monoclonal antibodies. CH-rPfs25 formulated in several adjuvants elicited strong immunogenicity in pre-clinical studies in mice. Immunization induced high titer of antibodies recognizing native Pfs25 on the surface of live gametes of *Plasmodium falciparum* in immunofluorescence assays, and the antibodies demonstrated complete transmission blocking activity in mosquito membrane feeding assays. The transmission blocking efficacy was dose dependent - 100% blocking even after 1:128 dilution of sera from mice immunized in complete Freund’s adjuvant or Montanide ISA51 and 1:16 dilution of sera from the Alum group. The blocking was mediated by antibodies and concentration as low as 31.25 µg/ml of total purified IgG exhibited 100% transmission blocking in *Anopheles gambiae* and *An. stephensi* mosquitoes. This study provides first ever evidence for successful expression and purification of functional rPfs25 in *E. coli* with extremely potent transmission blocking activity. Our results suggest that CH-rPfs25 expressed in *E. coli* can be further developed into vaccine formulations for evaluation in larger animals including human clinical trials. An effective transmission-blocking vaccine will play a central role in the goal of gradual malaria elimination and eventual eradication.
LIVER INDUCED IMMUNOTOLERANCE BY SALMONELLA-SPECIFIC CD4+ T-CELLS DURING CHRONIC INFECTION

Kurtz, Jonathan R* & McLachlan, James B.*

*Department of Microbiology & Immunology, Tulane University School of Medicine, New Orleans, Louisiana, USA

Background: *Salmonella* spp., a genus of rod-shaped Gram-negative enterobacteriaceae, causes a range of disease in humans and animals, such as typhoid fever, paratyphoid fever, and foodborne illnesses. In mice, *S.* Typhimurium causes a persistent bacterial infection analogous to typhoid fever in humans. Although a strong cellular immune response is initiated, the bacteria are never fully eliminated. It is currently unknown what mechanisms govern this immunological "stalemate." While it is known CD4+ helper T cells are essential effectors during *Salmonella* infection, it is not clear how these cells respond in infected tissues and what role tissue microenvironments play in directing immune responses. Our lab aims to illuminate the mechanisms that dictate CD4+ T-cell responses generated against *Salmonella* infection, especially during the chronic phase, and how host and microbial factors contribute to bacterial persistence.

Results: Our lab uniquely combines a murine model of persistent *S.* Typhimurium infection with recombineered *Salmonella* strains and MHC-II tetramers to visualize endogenous *Salmonella*-specific CD4+ T cell responses during infection in various organs over time. We show *Salmonella*-specific CD4+ T-cells adoptively transferred from lymphoid organs protect mice from subsequent *Salmonella* challenge, while FoxP3- CD4+ T-cells enriched and transferred from infected livers increase susceptibility to challenge, and that this phenomenon is dose dependent. Furthermore, lymphoid *Salmonella*-specific Th1-cells produce higher levels of the potent inflammatory cytokine interferon-γ, while FoxP3- liver CD4 T cells produce larger amounts of the immunosuppressive cytokine interleukin-10 and a decreased proliferative capacity. Additionally, we show lymphoid Th1 cells reduce macrophage bacterial loads, while liver-derived Tr1-like cells fail to control bacterial replication, possibly through attenuated iNOS activation. Whether these immunosuppressive Tr1-like cells are induced in the liver, or phenotypically switch upon entering the organ, remains under investigation. However to date, we show liver Kupffer cells isolated from chronically infected mice are capable of inducing proliferation of naïve T cells.

Conclusions: These results demonstrate that during persistent *Salmonella* infection different immunological responses occur at different anatomical sites, which may dictate the outcome of infection. Additionally, we have shown that the liver induces a more tolerogenic immune response than the lymphoid organs during chronic infection. Therefore, we hypothesize the liver may provide a privileged niche for *Salmonella* survival in vivo.

This work was supported by grants from the Louisiana Board of Regents (LEQSF(2012-15)-RD-A-24) and the National Institutes of Health (1 R01 AI103343-01A1).
HIV PREVALENCE AND BEHAVIORAL RISKS AMONG THE HOMELESS AND MARGINALLY-HOUSED IN NEW ORLEANS

Kwak M*, Olsen J **, Andrinopoulos K*

* Tulane University School of Medicine and Public Health, New Orleans, Louisiana.
** NO/AIDS Task Force, New Orleans, Louisiana.

**Objective**: To measure the prevalence of HIV and to describe the HIV-related behavioral risks in a growing, at-risk homeless and marginally-housed community in New Orleans to better plan effective programs for HIV prevention and management.

**Background**: Homelessness has increased in New Orleans since Hurricane Katrina in 2005. In the 2012 Homeless Point in Time (PIT) Count for New Orleans and Jefferson Parish, the number of those homeless or unstably housed was 2.4 times greater than the population estimate before the storm, even though the population back to the area is still only about 80%. Unstable housing is a serious issue in the HIV/AIDS epidemic for both the spread of HIV and ineffective medical intervention to prevent AIDS-related morbidity and mortality. The rate of new HIV diagnosis in New Orleans also increased during this same time period. Currently, there is no data reported on the prevalence of HIV in the homeless or unstably housed communities of New Orleans.

**Methods**: A retrospective chart review was conducted among homeless and marginally housed clients of two HIV testing services in New Orleans performed by the NO/AIDS Task Force (NATF) and the Tulane University Weekend Clinic at Ozanam Inn (TUOZ) between January 2008 to November 2013. Participants were recruited by street outreach, fliers, and referrals by peers and healthcare providers. HIV testing services included information such as year of birth, gender, race, intravenous drug use, and sexual risk behaviors. Linkage to care information was collected from NATF and the Louisiana Office of Public Health (LOPH). Data analysis was performed using Stata 12 software. The protocol for this study was approved by the Tulane University Institutional Review Board, reference number 13-240863E.

**Results**: A total of 302 participants sought testing and met inclusion criteria. The majority were African-American (223, 73.8%) men (236, 78.2%) with average age of 42+/-12.6. Most were tested at shelters (176, 58.3%) compared to the street (79, 26.2%) or homeless resource centers (40, 13.3%). However, most HIV preliminary positive participants were tested on the street (4/8, 50%) and were more likely to be linked to care (2, 33.3%) compared to the other testing locations. Overall preliminary positive rate in the homeless cohort was 2.65%, vs the NATF rate in 2011 of 2.5%.

**Conclusion**: The overall preliminary positive rate in the homeless and marginally housed was greater even in focused HIV at-risk testing performed by NATF. This shows that the homeless population in New Orleans is at increased risk of being HIV positive and spreading it to others.
Hedgehog (Hh)-signaling plays a critical role in liver development and repair process to liver injury. Recent evidence has indicated that hepatic Hh-signaling is highly activate in nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) patients. However, the role of the Hh signaling pathway in NAFLD and NASH has not been explored. In this study, we investigated the effect of Hh-signaling inhibition in high fat-induced hepatic steatosis \textit{in vitro} and \textit{in vivo}. First, we observed that Hh inhibition significantly reduced the lipid accumulation in the cultured mouse hepatocytes by staining of lipid droplets with Oil red O and BODIPY. Furthermore, Hh inhibitors also markedly decreased expression of SREBP1, which is a master regulator of lipid biosynthesis. For the \textit{in vivo} study, C57BL/6 mice were fed with chow or high-fat diet for 10 weeks. Three weeks prior to sacrifice, mice were treated with Hh inhibitors, GDC-0449, LED225 and GANT61. High-fat diet fed mice showed significantly increased expression of SREBP1 and its target gene Stearoyl-CoA desaturase-1 (SCD-1). However, high-fat diet fed mice with Hh inhibitors showed downregulation of SREBP1 and SCD-1. In addition, treatment with Hh inhibitors decreased serum cholesterol levels and improved glucose tolerance test compared to high-fat diet fed mice with vehicle treatment. Interestingly, fat accumulation and serum triglyceride levels were not changed by Hh inhibitors. In summary, inhibition of Hh signaling regulates genes involved in lipid metabolism and may contribute to the improvement of glucose homeostasis.
RISK OF BRCA MUTATION IN LOUISIANA PATIENTS WITHOUT ACCESS TO GENETIC TESTING

Langston JA*, Dvorak CT**, Andersson HC**

*Tulane University School of Medicine and Epidemiology Department of the Tulane University School of Public Health, New Orleans, LA
**Hayward Genetics Center, Tulane University, New Orleans, LA

Prophylactic interventions based on knowledge of BRCA1 and BRCA2 mutation status are proven to be directly related to decreased cancer incidence. However, in our experience at the Tulane Hayward Genetics Center in Louisiana, a large percentage of counseled patients do not complete the BRCA mutation genetic testing which would allow them to pursue these options. The purpose of this study is to (1) assess the performance of the BRCAPro risk of mutation model in our population and (2) identify patient demographics and predicted mutation carrier risk for those patients who have not completed genetic testing as they compare to those patients who did complete testing in order to better define the lost opportunity for intervention that these patients represent. Patient records from those counseled on BRCA1/2 testing between 1996 and 2013 at the Hayward Genetics Center at Tulane University were reviewed. For each patient, a family pedigree was created and used to apply the BRCAPro predictive model of BRCA1/2 deleterious mutation carrier risk. For those who did receive genetic testing, actual testing results were compared to predicted risk of mutation according to the BRCAPro model. Demographic characteristics were then compared between those patients who did versus those who did not complete genetic testing using the student’s t-test or chi-squared calculations as appropriate. After elimination of those patients with previously identified breast-cancer associated familial mutations, those whose charts could not be located, and those whose charts failed to include full pedigree information, 116 patients were identified, 78 of whom had completed BRCA1/2 testing and 38 who had not. Patients who did not receive testing were less likely to be white (29% vs. 54%, p=0.0115) and more likely to have Medicaid insurance (24% vs. 4%, p=0.0010). However, they had an equivalent risk of BRCA1/2 deleterious mutation carrier status according to BRCAPro calculations (0.0820 vs. 0.1197, p=0.2553), and an equivalent prevalence of previously diagnosed breast cancer (47% vs. 58%, p=0.2948). Analysis of the BRCAPro model revealed that while the model provides some population-level information on risk of mutation, it contained too much variability for reliable use on individual patients in our population. Access to BRCA1/2 testing among our population is heavily skewed away from minorities, particularly those who are black, and away from those with Medicaid insurance, despite the fact that these populations have similar risk profiles. This lack of genetic testing cannot be offset by modeling of risk, as the most commonly used model in our center has been shown to be insufficiently reliable to the individual in our population. This demonstrates a significant lost opportunity for intervention.
THE THERAPEUTIC POTENTIAL OF SECRETED ANTIVIRAL ENTRY INHIBITOR (SAVE) PEPTIDES EXPRESSED BY TRANSDUCED MSCs TO BLOCK HIV INFECTION


Biomedical Science Program*, Tulane National Primate Research Center**, Department of Pharmacology***, Tulane Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine****, Division of Virology, Innsbruck Medical University*****.

Infection with human immunodeficiency virus (HIV) results in CD4+ T cells depletion and the subsequent loss of immune function has led to the death of over 25 million people from AIDS. The population of people living with human immunodeficiency virus (HIV) has increased, mainly due to antiretroviral therapy. While the annual number of new HIV infections has remained relatively stable, the pace of new infections continues at far too high a level. In combination, antiretroviral therapies control viremia; however, drug regimens are complex and expensive, require life-long intervention with potential side effects. We have previously shown that expression of the membrane-associated and secreted C46 peptides, members of the new fusion inhibitor class of antiretroviral drugs, efficiently block infection of new cells by interfering with the function of HIV-1 gp41. To evaluate the potential therapeutic role of the Secreted Anti-Viral Entry inhibitory (SAVE) peptide in transduced mesenchymal stem cells (MSCs), we measured the inhibition of HIV infection in vitro with C46-transduced MSCs. First, we transduced human and rhesus BMSC (bone marrow-derived) with retroviral and lentiviral vectors (LV) expressing GFP (LZRS-GFP: murine leukemia virus, [MLV] and HRST-GFP [LV]), membrane-bound C46 (M218: MLV), or the secreted C46 (T-60: MLV and T-42: LV). Fluorescent microscopy and flow cytometry demonstrated that up to 69% of LV-transduced MSCs and 293T control cells expressed GFP. Molecular analysis of MLV ψ packaging element revealed that up to 25.5% of the human MSCs, rhesus MSCs, and 293T cells were transduced with the T-60 and M218 vectors. C46 was detectable in SAVE-transduced MSCs by western blot using 2F5 antibody. To conduct single round infection assay we propagated pseudo HIV particles with packaging vector (tat, rev, gag-pol), envelope vector (HIV envelope (JRFL) or pCMV-ΔR8.91), and transfer vector (HRST-GFP). After transient transfection, viral supernatant was collected and precipitated by PEG-it solution to concentrate. We sequentially conducted the single round infection assay to measure the inhibition of viral infection in vitro with conditioned medium from the SAVE-transduced MSCs. The data showed that conditioned medium from C46 and SAVE transduced rhesus BMSC blocked the infection of the HIV vectors by from 60-75%. In order to test whether transduction and the insertion of the transgene affect the differentiation of MSCs potency, we have conducted osteogenic, adipogenic, and chondrogenic differentiation assay on SAVE-transduced rhesus BMSCs. As a result, the entire transduced rhesus BMSCs maintained differentiation potential. Thus, SAVE peptides expressed by MSCs may provide an alternative in vivo drug delivery system to AIDS patients and, in combination with other anti-retroviral therapies, provide long term viral inhibition clinical efficacy.

This work was supported by a fellowship from the Tulane National Primate Research Center.
SINGLE AND JOINT ASSOCIATIONS OF GENETIC VARIANTS IN THE SERUM/GLUCOCORTICOID REGULATED KINASE (SGK) GENES WITH BLOOD PRESSURE RESPONSES TO SODIUM INTAKE: THE GENSALT STUDY

Changwei Li\textsuperscript{a}, Xueli Yang\textsuperscript{b}, Jiang He\textsuperscript{a,c}, James E. Hixson\textsuperscript{d}, Dongfeng Gu\textsuperscript{b}, Dabeeru C. Rao\textsuperscript{e}, Lawrence C. Shimmin\textsuperscript{d}, Jianfeng Huang\textsuperscript{b}, Charles C. Gu\textsuperscript{e}, Jichun Chen\textsuperscript{b}, Jianxin Li\textsuperscript{b}, Tanika N. Kelly\textsuperscript{a}

\textsuperscript{a} Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112, USA.
\textsuperscript{b} State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center of Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China.
\textsuperscript{c} Department of Medicine, Tulane University School of Medicine, New Orleans, LA, USA.
\textsuperscript{d} Department of Epidemiology, Human Genetics and Environmental Sciences, University of Texas School of Public Health, Houston, TX, USA;
\textsuperscript{e} Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; Campus Box 8067, 660 South Euclid Ave. St. Louis, MO 63110-1093, USA

*Contribute equally.

\textbf{Background}: Serum and glucocorticoid regulated kinase (SGK) plays a critical role in the regulation of renal sodium transport. We examined the association between SGK genes and salt sensitivity of blood pressure (BP) using single-marker and gene-based association analysis.

\textbf{Methods}: A 7-day low-sodium (51.3 mmol sodium/day) followed by a 7-day high-sodium intervention (307.8 mmol sodium/day) was conducted among 1,906 Chinese participants. BP measurements were obtained at baseline and each intervention using a random-zero sphygmomanometer. Additive associations between each SNP and salt-sensitivity phenotypes were assessed using a mixed linear regression model to account for family dependencies. Gene-based analyses were conducted using the truncated p-value method. The Bonferroni-method was used to adjust for multiple testing in all analyses.

\textbf{Results}: In single-marker association analyses, SGK1 marker rs2758151 was significantly associated with diastolic BP (DBP) response to high-sodium intervention (P=0.0010). DBP responses (95% confidence interval) to high-sodium intervention for genotypes C/C, C/T, and T/T were 2.04 (1.57 to 2.52), 1.79 (1.42 to 2.16), and 0.85 (0.30 to 1.41) mmHg, respectively. Similar non-significant trends were observed for SBP and MAP responses (P=0.15 and 0.0026, respectively). In addition, gene-based analyses demonstrated significant associations between SGK1 and SBP, DBP and MAP responses to high sodium intervention (P=0.0002, 0.0076, and 0.00001, respectively). Neither SGK2 nor SGK3 were associated with the salt-sensitivity phenotypes in single-maker and gene-based analyses.

\textbf{Conclusions}: The current study identified single-marker and gene-based association of the SGK1 gene and BP salt-sensitivity in the Han Chinese population. Further studies are warranted to identify causal SGK1 gene variants.

The Genetic Epidemiology Network of Salt Sensitivity (GenSalt) is supported by a cooperative agreement project grant (U01HL072507, R01HL087263, and R01HL090682) from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD.
Multicolour Fluorescence In-Situ Hybridization (M-FISH) imaging is used for detecting chromosomal rearrangements such as chromosomal translocations, deletions and inversions. Due to imaging noise and many other imaging problems, there is always a classification error, leading to wrong detection of chromosome abnormalities. Therefore, how to accurately classify chromosomes from M-FISH images becomes a challenging problem. In this paper we propose a structure based sparse model with different constraints by extending the general sparse model to the multiple pixels case, where each pixel together with its neighboring pixels are used simultaneously in the sparse representation of chromosome classes. We use the model to classify multicolor fluorescence in-situ hybridization (M-FISH) images. Both the simulation and real data analysis results show that the structure based sparse model penalized with lp norm (p=0 and p=1) improved the accuracy of classification over the conventional sparse model based classifier, which translates into improved diagnosis of genetic diseases and cancers.
CHILDHOOD SECONDHAND SMOKING IS ASSOCIATED WITH ADULT OBESITY MEASURES ONLY IN WOMEN: THE BOGALUSA HEART STUDY

Li S*, Fernandez CA*, Chen W*, Srinivasan SR*, Berenson GS*

*Tulane Center for Cardiovascular Health and Department of Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, LA

Secondhand smoking (SHS) is an important risk factor for cardiometabolic diseases. It is unknown whether exposure to SHS is associated with obesity during childhood, and if so, whether such an association persists into adulthood. These aspects were examined in a community-based, black-white sample (n=752, 33.4% females) from the Bogalusa Heart Study, with an average follow-up period of 25.8 years. Standardized body mass index (BMI) scores by age were used during childhood (age range: 4.4-17.9 years; mean age 9.6 years), and BMI and waist to height ratio (WHtR) were used as obesity measures during adulthood (age range: 18.0-50.9; mean age 36.2 years). Information on SHS during childhood was obtained by a self-administered questionnaire. General linear models were used to examine the effects of SHS on obesity measures. Girls (age<18 years) who were exposed to SHS had higher standardized BMI z-scores than girls who were not (0.02±0.07 vs. -0.23±0.06, least square means±SE, adjusted for race; p=0.006); such a difference was not observed in boys (age<18 years) (-0.01±0.09 vs. 0.14±0.08, p=0.22). During adulthood (age≥18 years), women who were exposed to SHS during childhood had higher BMI and WHtR than those who were not (30.9±1.0 kg/m² vs. 29.5±0.9 kg/m², p=0.048 for BMI and 0.50±0.02 vs. 0.48±0.02, p=0.036 for WHtR; adjusted for age and race for both); such differences were not observed in men (28.9±1.0 vs. 29.7±1.0 kg/m² for BMI, p=0.27; 0.52±0.02 vs. 0.53±0.02 for WHtR, p=0.24). After adjusting for childhood standardized BMI z-scores, the differences in adult BMI and WHtR between those who were exposed to SHS during childhood and those who were not were no longer significant in women (p=0.93 and p=0.78, respectively). In conclusion, girls exposed to SHS have increased BMI, and such an effect persists into adulthood. These results underscore the importance of protecting children, particularly girls from exposure to SHS.

The study is supported by grants 5R01ES021724 from National Institute of Environmental Health Science and 2R01AG016592 from the National Institute on Aging. Shengxu Li is a scholar of the Building Interdisciplinary Research Careers in Women’s Health (BIRCWH) program, supported by Award Number K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development.
High blood pressure (BP) is a complex trait, influenced by multiple environmental and genetic determinants. Although established as a heritable trait, the genomic mechanisms underlying BP regulation remain largely unknown. The objective of the current study was to examine the associations of endothelial system genes with BP changes and hypertension incidence among 1,775 Han Chinese participants of the family-based Genetic Epidemiology Network of Salt Sensitivity (GenSalt) follow-up study. Nine BP measurements were obtained at baseline and during each of two follow-up observations using a random-zero sphygmomanometer. The associations of 206 SNPs in 15 endothelial system genes with BP changes and hypertension incidence were assessed using mixed models to account for the correlations of repeated measures among individuals and within families. A genotype by time interaction term was used to model differences in longitudinal BP change according to genotype over time. Gene-based analyses were conducted using the truncated product method. The Bonferroni method was used to adjust for multiple testing in all analyses. Among those free from hypertension at baseline, 513 (32.1%) GenSalt participants developed hypertension during the average 7.2 years of follow-up. In single-marker analyses, each copy of the minor allele of \( \text{SELE} \) markers rs4656704, rs6427212 and rs5368 was associated with increased risk of hypertension with relative risks (95% confidence intervals) of 1.42 (1.18, 1.71), 1.46 (1.21, 1.75) and 1.46 (1.21, 1.77), respectively (\( P = 1.66\times10^{-4}, 7.44\times10^{-5} \) and \( 8.51\times10^{-5} \), respectively). \( \text{SELE} \) marker rs3917436 predicted longitudinal DBP change, and the average DBP changes were 1.14, 1.00, and 0.80 mmHg in participants with G/G, G/A and A/A genotype, respectively (\( P_{\text{for trend}} = 8.27\times10^{-5} \)). Results of gene-based analyses showed the \( \text{SELE} \) gene was significantly associated with SBP change, DBP change and hypertension incidence (all \( P<1.00\times10^{-6} \)). \( \text{EDNRA} \) gene was significantly associated with hypertension incidence (\( P=2.00\times10^{-4} \)), and \( \text{DDAH1}, \text{SELP} \text{ and COL18A1} \) genes were associated with SBP change (\( P=2.00\times10^{-6}, 9.00\times10^{-5} \) and \( 1.00\times10^{-6} \), respectively). In conclusion, the current study provides strong evidence of the association of \( \text{SELE} \) markers rs4656704, rs6427212, rs3917436 and rs5368 with BP progression. Furthermore, gene-based analyses support roles of \( \text{DDAH1}, \text{SELP}, \text{SELE}, \text{EDNRA} \) and \( \text{COL18A1} \) genes in BP regulation. Future studies will be required to identify the causal variants underlying the observed associations.

This study is supported by research grants (R01HL087263 and R01HL090682) and training grant (D43TW00917) from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.
The development of castration resistant prostate cancer (CRPC) following androgen deprivation therapy remains the most critical challenge in the clinical management of prostate cancer. Mounting evidence suggests that sustained AR activation, sometimes in a ligand-independent manner, is central to the development of CRPC. AKT, a key survival pathway in prostate cancer, has also been shown to promote CRPC. Recently, it has been shown that AR and AKT crosstalk by reciprocal inhibition. These studies suggest that treatment approaches targeting one of these pathways in CRPC could lead to derepression of the other, leading to therapeutic resistance. This underscores the urgent need for novel agents and strategies co-targeting AR and AKT in CRPC. Preliminary studies in our lab demonstrate that berberine (BBR) suppresses the expression of AR and AKT in vitro and in tumor xenografts. In the current study, we evaluate the efficacy of BBR in a transgenic model and investigated the mechanisms of AR and AKT downregulation. The in vivo efficacy of BBR was evaluated in the Pten knockout mouse models, at a dose of 5 mg/kg/day. The modulation of AR and AKT expression was determined in the excised tumor specimens by immunohistochemistry and Western blot analysis. Quantitative RT-PCR was performed to determine the effect of BBR on mRNA expression of AR and AKT. The influence on the protein stability of AR and AKT was evaluated by determining the half-lives of AR and AKT and by in vitro ubiquitinylation assays. We found that BBR inhibited prostate tumorigenesis in Pten-null mice, which express high levels of activated AKT. The levels of AR and AKT were reduced by BBR in the malignant tissues, but not in the normal tissues, suggesting BBR exerts its effects on AR and AKT in a tumor-specific manner. Additionally, BBR inhibited tumor recurrence after castration. Mechanistically, BBR inhibited AR expression by inhibiting AR mRNA transcription and inducing AR protein degradation. In contrast, the effect of BBR on AKT expression was mainly through inducing protein degradation and this process was mediated by the production of reactive oxygen species. In summary, our study suggests that BBR inhibits prostate cancer growth and castration-resistant progression through co-targeting the AR and AKT pathways.
ATTENUATED MONOCYTE APOPTOSIS, A NEW MECHANISM FOR OSTEOPOROSIS SUGGESTED BY A TRANSCRIPTOME-WIDE STUDY OF MONOCYTES.


*Department of Biostatistics and Bioinformatics, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA, 70112

Background: Osteoporosis is caused by excessive bone resorption (by osteoclasts) over bone formation (by osteoblasts). Monocytes are important to osteoporosis by serving as progenitors of osteoclasts and produce cytokines for osteoclastogenesis.

Objective: To identify osteoporosis genes by microarray analyses of monocytes in high vs. low hip BMD (bone mineral density) subjects.

Method: Microarray analyses of monocytes were performed using Affymetrix 1.0 ST arrays in 73 Caucasian females (age: 47-56) with extremely high (mean ZBMD =1.38, n=42, 16 pre- and 26 postmenopausal subjects) or low hip BMD (mean ZBMD=-1.05, n=31, 15 pre- and 16 postmenopausal subjects). Differential gene expression analysis in high vs. low BMD subjects was conducted in the total cohort as well as pre- and postmenopausal subjects. Focusing on the top differentially expressed genes identified in the total, the pre- and the postmenopausal subjects (with a p <5E-03), we performed replication of the findings in 3 independent datasets of microarray analyses of monocytes in: 1) 80 Caucasian females (40 high vs. 40 low BMD subjects); 2) 19 Caucasian females (10 high vs. 9 low BMD subjects); and 3) 26 Chinese females (14 high vs. 12 low BMD subjects).

Result: We identified (in the 73 subjects) and successfully replicated in all the 3 independent datasets 2 genes, DAXX and PLK3. Interestingly, both genes are apoptosis induction genes and both down-regulated in the low BMD subjects. In addition, among the list of genes (n = 102) that are replicated in the largest replication cohort containing the 80 subjects, 2 other genes (PDCD5 and VDAC1) that are ranked at the top (7th and 17th, respectively, based on meta-analysis p values) are also apoptosis induction genes and down-regulated in the low BMD subjects. Overall, our result may suggest that there might be a lower apoptosis activity of monocytes in the low BMD subjects.

Conclusion: Our study for the first time suggested a lower apoptosis rate (hence an increased survival) of monocytes, an important osteoclastogenic cell, as a novel mechanism for osteoporosis.

This work was supported by NIH grants.
The cardiac hormone atrial natriuretic peptide (ANP) regulates blood pressure and blood volume by activation of guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) and subsequent generation of intracellular second messenger cGMP. The objective of this study was to visualize the internalization and trafficking of NPRA in the subcellular compartments of intact cells. We utilized immunofluorescence staining and co-immunoprecipitation (co-IP) of plasma membrane, endosomal, lysosomal, and Rab 11 markers to follow trafficking and signaling by confocal immunofluorescence microscopy and immunoblotting. A chimeric construct of enhanced green fluorescence protein (eGFP) and NPRA (eGFP-NPRA) was used to study internalization and trafficking of receptor in human embryonic kidney-293 (HEK-293) cells. The treatment of cells with ANP at different times accelerated the internalization of receptor from cell surface to cell interior. Colocalization of eGFP-NPRA with pan-Cadherin indicated that internalization of receptor accounted for 58.4%, 69.0%, 70.8%, and 75.6% at 5, 10, 15, and 30 min, respectively, compared with untreated cells. Colocalization of eGFP-NPRA with early endosome antigen-1 (EEA-1) marker was maximum at 5 min (63.3%), and gradually decreased at 10 min (42.8%), 15 min (30.9%) and at 30 min (23.2%) respectively. Similarly, colocalization of NPRA with lysosome-associated membrane protein-1 (LAMP-1) gradually increased at 5 min (13.0%), 10 min (35.6%), 15 min (42.5%), and at 30 min (44.5%). Rab 11 was used as a recycling endosome marker, which showed that 20% receptor recycled back on plasma membrane. Co-IP assays confirmed, in a biochemical context, the interaction of NPRA with these organelles during endocytic process. Immunofluorescence and enzyme-linked immunosorbent assay (EIA) analyses showed a marked increase in the accumulation of intracellular cGMP concurrent with receptor internalization. Our study suggests that after ligand binding, receptor internalized and trafficked into subcellular compartments with concurrent generation of intracellular cGMP, which play critical role in the regulation of hypertension and cardiovascular homeostasis.

This work was supported by the NIH grant (HL57531).
Abstract

The main objective of this research was to identify commonly misconstrued rape myths prevalent on campuses through the student perceptions of sexual assault at Tulane University and to juxtapose perceptions with multi-study confirmed data that reveals the largely unknown truth about the nature of university rape. The research shows that the victims of sexual assault do not trust our society enough to report the crime to us. In the few instances where the victim does come forward, the young men in question are often protected by authorities who believe in second chances when in fact they are not one time offenders, but rather predators. The compilation of data provides a comprehensive overview of modern day understanding of rape of female college students. Combined with the perceptions and suggestions of the students and staff members of Tulane, the data provides a powerful outlook for potential change and improvement starting with the raised awareness of populations regarding the most powerful rape myths uncovered in this research.
REVERSION-INDUCING CYSTEINE-RICH PROTEIN WITH KAZAL MOTIFS (RECK) PLAYS A PROTECTIVE ROLE IN NEOINTIMAL HYPERPLASIA


*Research Service, Southeast Louisiana Veterans Health Care System, New Orleans, LA
**Heart and Vascular Institute, ***Medicine/Gastroenterology and Physiology, Tulane University School of Medicine, New Orleans, LA
****Medicine, University of Texas Health Science Center and South Texas Veterans Health Care System, San Antonio, TX
#Corresponding Author, Tel.: 504-988-3034, E-mail: bchandra@tulane.edu

Neointimal hyperplasia is characterized by the sustained induction and activation of matrix-degrading MMPs, ECM destruction, and smooth muscle cell (SMC) migration and proliferation. RECK (Reversion-inducing cysteine-rich protein with Kazal motifs) is a unique membrane-anchored glycoprotein, and an inhibitor of MMPs 2, 7, 9, and 14. To date, its expression and role in neointima formation/progression is not known. We hypothesized that RECK downregulation and MMP induction contribute causally to neointima formation. We used two models of neointimal hyperplasia; collar injury-induced hyperplasia in carotid artery (perivascular, 3 weeks) and wire injury-induced hyperplasia in femoral artery (intraluminal, 2 weeks). Both collar and wire injury induced neointima formation (increased intima/media [I/M] ratio and intimal area) in 3 month-old, wild type, C57Bl/6 mice. No hyperplastic response was observed in the respective contralateral control vessels. RECK expression was markedly downregulated in the hyperplastic vessels. However, MMP expression (2, 7, 9 and 14) was upregulated, implying that RECK downregulation may contribute to enhanced MMP expression, and SMC migration and proliferation. In transgenic (Tg) mice that overexpress RECK in SMC-specific manner (SM22αGC promoter; SMC-RECK Tg), neointima formation was significantly reduced compared to littermate controls, independent of type and site of injury. The percent decrease in I/M ratio and intimal area was ~38-40 and ~47-50 (p=0.0048 and 0.029) respectively in the collar injury model, and ~41-44 and ~49-52 (p=0.008 and 0.0219) respectively in the wire injury model. These results indicate that RECK downregulation and MMP overexpression contributes causally to neointima formation. Further, RECK overexpression blunts this hyperplastic response. Therapeutic strategies that upregulate RECK might inhibit progression of the hyperplastic vascular diseases such as atherosclerosis, restenosis, transplant vasculopathy and vein bypass graft failure.
PROPHYLACTIC TARGETING OF THE NEUROKININ-1 RECEPTOR ON NEUROINFLAMMATION IN A EX VIVO MODEL OF LYME NEUROBORELIOsis

Martinez AN*, Ramesh G*, Philipp MT*

*Division of Bacteriology & Parasitology, Tulane National Primate Research Center, Covington, LA.

Inflammation caused by the Lyme disease spirochete *B. burgdorferi* (*Bb*) is an important factor in the pathogenesis of Lyme neuroborreliosis. The neuropeptide substance P (SP) and its specific receptor, neurokinin-1 receptor (NK-1R), play an important role in inflammation and neurotoxic responses of glial cells to clinically important bacterial pathogens of the central nervous system (CNS). Thus, the goal of this study was to test if inhibition of SP/NK-1R interactions attenuate inflammatory immune responses and neuronal damage in an *ex vivo* nonhuman primate (NHP) cortical brain explant culture model of *Bb* CNS infection. Results from multiplex ELISA cytokine assay showed that *Bb* elicited elevated levels of IL-6, IL-8 and CCL2 in the brain tissues. Prophylactic NK-1R antagonist treatment limited elevations of these inflammatory mediators, which indicates that therapeutic intervention may attenuate neuroinflammation. Concomitant apoptosis was quantified and both neurons and oligodendrocytes were found to be undergoing apoptosis. However, the percentage of apoptotic neurons was low (less than 2%) as compared to oligodendrocytes (30.6 %) and in the presence of NK1R antagonist only oligodendrocytes showed a statistically significant reduction in the number of *Bb*-induced apoptotic cells. Our results suggest that dampening pro-inflammatory cascades to limit unchecked neuroinflammation and subsequent oligodendrocyte damage by NK1R antagonist may be beneficial to LNB patients. As such, the demonstration that inhibition of SP/NK-1R interactions ameliorates acute bacterially-induced CNS damage in NHP cortical brain tissue is a significant step in demonstrating the therapeutic potential of such an approach in the treatment of meningitis and, potentially, other inflammatory CNS conditions.

This work was supported by grant **R01 NS 050325** from the NIH.
NOVEL KINASE PATHWAYS IN THE REVERSAL OF EPITHELIAL-MESENCHYMAL TRANSITION IN AGGRESSIVE BREAST CANCERS

Matossian MD*, Elliott S*, Collins-Burow BM*, Burow, ME*

*Department of Medicine: Section of Hematology and Oncology, Tulane University, New Orleans LA

Abstract: Breast cancer has a high mortality rate in women, second only to lung cancer. 90% of breast cancer mortalities are due to metastasis. Epithelial-mesenchymal transition (EMT), the loss of an epithelial cell phenotype and acquisition of a mesenchymal cell phenotype, has been implicated in the progression of breast cancer cells to an invasive, metastatic phenotype. Thus the development of novel agents that can regulate EMT may represent an important approach for suppression of cancer metastasis. Previous research has demonstrated the role of various cell signaling pathways in regulating the EMT axis, including cytokine and growth factor receptors as well as downstream kinases such as the mitogen-activated protein kinase family. While work has focused on defining the mechanisms by which these pathways regulate EMT, these kinases represent only a small sample of the human kinome that has been investigated in regulating tumorigenesis and metastasis. To identify additional kinase pathways that play a role on regulating cancer cell EMT we utilized a small molecule kinase inhibitor screen approach. We employed a library of 875 compounds provided by GlaxoSmithKline (GSK) consisting of intermediate and terminal synthesis compounds representing inhibitors across a number of known kinases and therapeutic targets (polo-like kinase, c-Jun N-terminal kinase, protein kinase B, insulin-like growth factor, for example). We hypothesized that we would find groups of inhibitors to specific kinases that exhibited reversal of the EMT phenotype. Our lab treated three aggressive metastatic breast cancer cell lines with a mesenchymal phenotype for three days, followed by crystal violet staining and microscopy visualization of morphology. Our initial screen in MDA-MB-231 cells revealed 36 hits across 12 major kinase categories (including polo-like kinase, anti-malarial drugs, VEGFR, and AKT/protein kinase B). Follow up screen in two additional cell lines MCF-7FR and MCF-7-CXCR4-CTD revealed twenty kinase inhibitor compounds that appeared to have the most significant EMT reversal in all three cell lines. While a number of interesting individual inhibitors with activity have emerged, some polo-like kinase (PLK) inhibitors exhibited activity in all three cell lines. PLK has been shown to be involved in tumor invasion in some cancer types, including breast cancer, but no reports of its effects on EMT have been published. Subsequent work will focus on analysis of changes in EMT markers, such as E-cadherin and Vimentin, with selected compounds. Future directions will elucidate mechanisms behind the kinase pathways that drive the most significant changes in EMT reversal and to lyse and quantify the stained cells to determine proliferation of the treated cells. Overall, we believe this small molecule kinase inhibitor screen represents a powerful tool to identify a potential therapeutic targets for very aggressive metastatic breast cancers. This research was supported by the National Institutes of Health, National Cancer Institute R01CA125806-01A2.
THE EFFECTS OF OSCILATORY FLOW ON MOUSE STEM CELL DIFFERENTIATION

*Messina SL, *Ahsan T
*Department of Biomedical Engineering, Tulane University, New Orleans, LA

Because of their ability to mature from an unspecialized state to a differentiated state, stem cells have created new opportunities for regenerative medicine. Differentiation of embryonic and adult stem cells can be controlled through many mechanisms including a variety of biological growth factors and applied mechanical forces that result in shear stress, compression, and tension. Recent stem cell mechanobiology studies have shown the effects of steady laminar fluid shear stress on early stem cell differentiation. While linear flow promotes mesoderm differentiation and hematopoietic phenotypes, the effect of varying profiles is still largely unknown. This study will expand upon current knowledge by showing how oscillatory and pulsatile flow at various frequencies affects mouse embryonic stem cell (ESCs) differentiation. ESCs were cultured on collagen type IV coated glass slides. With the use of a modified parallel plate bioreactor, the two-dimensional samples were placed under specific fluid shear stresses. Flow profiles were designed using the StreamSoft computer program. Samples were analyzed and germ specification was determined using PCR arrays. These studies are expected to show differences in stem cell fate depending on flow rate and flow type. This will further the understanding of how physical cues within the stem cell's microenvironment affect stem cell fate. Once ESC effects are classified, additional research can be done using human induced pluripotent stem cells (iPSCs).
Major depressive disorder will affect one in six Americans. Traditional pharmaceutical therapies offer significant clinical value but have serious drawbacks. A single sub-anesthetic dose of ketamine, a non-competitive NMDA receptor (NMDAR) antagonist, has been shown to induce rapid anti-depressant effects in human patients and rodent models within 30 minutes and lasting up to 14 days.

Ketamine treatment increases protein synthesis and promotes synapse formation in prefrontal cortex (PFC). We have previously shown that signaling via GluN2B subunit-containing NMDARs suppresses protein synthesis in cortical neurons under basal levels of activity. We therefore hypothesized that in animals lacking GluN2B-containing NMDARs in cortex (2BΔCtx) an increased basal rate of translation will mimic and occlude the effects of ketamine.

In wild-type (WT) and 2BΔCtx adult mice we tested the effects of a single dose of ketamine on synaptic physiology of pyramidal neurons in layer II/III of the PFC. In acute brain slices from 2BΔCtx animals we observed an increase in basal excitatory synaptic activity, measured as an increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs). The ketamine induced increase in mEPSC frequency seen in control animals was mimicked and occluded in 2BΔCtx mice, indicating that antagonism of GluN2B-containing NMDARs is critical for ketamine's effects. Finally, we show that GluN2B-containing NMDARs are uniquely activated by ambient glutamate, and that by manipulating ambient glutamate levels (and thus tonic activation of GluN2B), we can modify expression of behavioral despair and synaptic physiology.

Based on these results, we propose a novel mechanism whereby ketamine exerts its effects by relieving tonic activation of GluN2B-containing NMDARs, resulting in enhanced translation and synaptic reorganization in the PFC.

This work was supported by a grant from the NIMH (MH099378-01) and a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation (YIA18996) to BJH. OHM was supported by a Louisiana Board of Regents Fellowship.
**URINARY SODIUM AND POTASSIUM EXCRETION AND CARDIOVASCULAR DISEASES IN PATIENTS WITH CHRONIC KIDNEY DISEASE: THE CHRONIC RENAL INSUFFICIENCY COHORT STUDY**


*Department of Epidemiology, Tulane University, New Orleans, LA; ** Department of Medicine, Tulane University, New Orleans, LA; *** Department of Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA; **** Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, MD; ***** Division of Kidney, Urologic, and Hematologic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD; ****** Department of Medicine, Temple University, Philadelphia, PA; ******* Department of Medicine, University of Pennsylvania, Philadelphia, PA; ******** Department of Internal Medicine, University of Michigan, Ann Arbor, MI; ********* Department of Medicine, Case Western Reserve University, Cleveland, OH; ********** Department of Medicine, University of Illinois, Chicago, IL; *********** Section of Hypertension, St. John Hospital and Medical Center, Detroit, MI

**Introduction:** Chronic kidney disease (CKD) patients are at an increased risk of cardiovascular disease (CVD) compared to the general population. Prior work has produced contradictory results for the associations of sodium and potassium intake with CVD incidence, and these associations have not been investigated in patients with CKD.

**Objectives:** To assess the prospective associations between urinary sodium and potassium excretion and CVD incidence among patients with CKD.

**Methods:** The Chronic Renal Insufficiency Cohort Study (CRIC) is a prospective cohort study of 3,939 participants with CKD from seven locations in the United States. Dietary sodium and potassium intake are averaged from three 24-hour urine samples, and the ratios of sodium and potassium to urinary creatinine are used. Composite CVD is defined as incident myocardial infarction (MI), stroke, or congestive heart failure (CHF). CVD events are reported every six months and confirmed by medical record adjudication.

**Results:** Over an average 6.5 years of follow-up, 660 CVD events occurred. The highest quartile (>199.5 mmol/1,374 mg) of the sodium-to-creatinine ratio had a hazard ratio (HR) of 1.58 (95% confidence interval 1.27, 1.97; \( p \) for trend across quartiles <0.0001) for composite CVD events compared to the lowest quartile (≤129.0 mmol/1,374 mg) after adjustment for important covariates. Similarly, the highest quartile (>69.8 mmol/1,374 mg) of the potassium-to-creatinine ratio had a HR of 1.61 (1.28, 2.03; \( p \) for trend across quartiles 0.0002) for composite CVD events compared to the lowest quartile (≤42.3 mmol/1,374 mg). When modeled continuously, every 100-mmol higher sodium to 1,374 mg creatinine ratio was associated with an increased HR of 1.23 (1.12, 1.35) for composite CVD events, 1.23 (1.11, 1.37) for CHF, 1.35 (1.11, 1.64) for stroke, and 1.12 (0.96, 1.32) for MI. In addition, every 50-mmol higher potassium to 1,374 mg creatinine ratio was associated with an increased HR of 1.27 (1.11, 1.44) for...
composite CVD events, 1.35 (1.17, 1.56) for CHF, 1.14 (0.82, 1.59) for stroke, and 1.03 (0.81, 1.32) for MI.

**Conclusion**: Our study found that dietary sodium and potassium are both associated with an increased risk of CVD among patients with CKD. These findings suggest that reductions in dietary sodium and potassium might reduce the incidence of CVD among patients with CKD.

This work was supported by a grant for the National Institute of Diabetes and Digestive and Kidney Diseases (NIH) R01 DK074615.
SOIL ANALYSIS OF URBAN FARMS IN NEW ORLEANS, LA


*Tulane University School of Public Health and Tropical Medicine, Department of Global Environmental Health Sciences, New Orleans, LA  
**Mary Queen of Vietnam Community Development Corporation, New Orleans, LA

Urban agriculture provides improved access to healthy food, stimulates local economic development, and fosters sustainable land use. However, urban soils in relatively old cities may have unsafe levels of contaminants that could impact the quality and safety of the produce grown in these areas. We are evaluating soil levels of trace elements, semi-volatile organic compounds, and diesel-range organics from urban farm sites in New Orleans, LA, to determine whether unsafe levels of contaminants are present. We have collected and analyzed primarily topsoil using a chain of custody approach from cooperative urban farm sites within the New Orleans metropolitan area. Trace elements were analyzed by ICP/MS (Hg CVAAS) and organic compounds were analyzed using GC/MS. Results were compared to the Louisiana Department of Environmental Quality's (LDEQ) soil standards. Results from one of our farm sites revealed that 98.9% of samples were below the LDEQ soil standards for the elements and compounds tested. One sample exceeded the standard for diesel range organics but this zone is not currently under production. Four samples were above the standard for iron, and one sample exceeded standards for iron and aluminum. Maintaining a soil pH greater than 6.5 in order to mitigate any effects from the excess iron and aluminum was provided as risk management advice. We conclude that the soil at this site is suitable for agricultural use and the produce from this site is likely safe for consumption. We are examining additional urban farm sites in areas with varying degrees of historical urbanization. Ultimately, our goal is to develop a policy and plan, from sampling to mitigation, for examining soils in highly urbanized areas where legacy pollution may create a foodborne hazard when converted to agricultural production regardless of scale.

This work is supported by the Baton Rouge Area Foundation.
Cytogenomic aberrations are common defects in patients with isolated Multicystic Dysplastic Kidney

D.J. Monlezun¹, T.J. Chen¹², A. Ramalingam¹², R. Song¹, A. Janssen¹², G. Preston¹², I.V. Yosypiv¹

¹Department of Pediatrics, and 2) Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA

PURPOSE OF STUDY: Several lines of evidence strongly suggest that genetic factors contribute to the pathogenesis of multicystic dysplastic kidney (MCDK). However, the pathogenic roles of cytogenomic aberrations for isolated MCDK has not been well investigated yet. We performed Array Comparative Genomic Hybridization (aCGH) in 10 children with isolated MCDK in order to reveal the pathogenic mechanisms underlying MCDK.

METHODS USED: Patients (six female and four males, mean age 8.5+/- 1.1 years) were diagnosed with MCDK by ultrasonography. All patients had normal renal function at the time of blood specimen collection. Genomic DNA was isolated from blood leukocytes and buccal cells. aCGH was performed on Agilent 105K array according to the manufacturer’s protocol. Data was analyzed using Cytogenomic software package from Agilent. To confirm the inherited pattern of the detected alteration, quantitative PCR (qPCR) was performed on patients and their respective parents for each detected aberration with reference control. The relative copy number was calculated by ΔΔCt method.

SUMMARY OF RESULTS: Three pathogenic aberrations were detected in three patients. The first aberration was a deletion at 7p14.3 with size of 2.07 Mb. At least 12 genes are located within this deletion region, including BBS9 and BMPER gene. The second aberration was a duplication on 16p13.11p12.3 with size of 3.28 Mb. There are more than 20 genes located within this duplicated region. The third aberration is a monosomy X for a female patient. qPCR demonstrated that deletion at 7p14.3 was inherited from patient’s father, while the duplication at 16p13.11-p12.3- from patient’s mother. The third aberration was monosomy X which resulted from meiotic nondisjunction in oogenesis or spermatogenesis. Its parental origin was not studied.

CONCLUSIONS: Our results demonstrate that 30% of MCDK patients possessed cytogenomic aberrations. Mutations in BBS9 and BMPER have been reported to result in cystic kidney dysplasia or diaphanospondylodysostosis with cystic kidneys, suggesting the pathogenic function for the deletion at 7p14.3. However, these two aberrations were inherited from parents, indicating that complex molecular mechanisms underlie MCDK in children. We conclude that: 1) Cytogenomic aberrations represent a common genetic defect in MCDK patients, and 2) aCGH is a valuable tool to reveal pathogenic mechanisms of MCDK in humans.
Racial disparities in pregnancy outcomes are well established. Inadequate calcium intake is known to be associated with many pregnancy outcomes and historically, calcium intake has differed by race. The goal of this study is to determine if racial disparities in calcium intake persist in more recent years and if these disparities differ in pregnant and non-pregnant women of child-bearing age. Total calcium intake was calculated from dietary and supplementation information using 4 survey cycles from the National Health and Nutrition Examination Survey data, from 2003 to 2010. Inadequate calcium was defined as intake less than 1,000 mg/day for both pregnant and non-pregnant women. The study included 7,267 women of childbearing age. Inadequate calcium intake ranged from 55% in 2005-2006 to 62% in 2003-2004 (no statistically significant differences). African American women were 3.8 (p<0.0001) and 2.0 (p<0.0001) times more likely to have inadequate calcium intake compared to whites during 2003-2004 and 2009-2010, respectively. Disparities remain among pregnant women: in most recent survey cycles, 83% and 76% of African-Americans had inadequate calcium intake, versus 26% and 21% of whites during pregnancy. Pregnant African-Americans were 3.3 (p=0.002) times more likely to have inadequate intake in 2009-2010, compared to pregnant whites. Racial disparities were not significant for Hispanic versus white women during. Consistent with historical data, racial disparities in calcium intake persist among both pregnant and non-pregnant women. More than half of women of child-bearing age have inadequate daily dietary and supplemental intake of calcium. Inadequate calcium intake remains high in pregnant African-Americans.
Malaria is a vector borne parasitic disease that kills over half a million children every year in Africa alone. In the absence of an efficacious vaccine, drugs are our only weapon to combat the disease. Unfortunately, drug resistant strains of *P. falciparum* -the leading parasite in human infections- are widely prevalent in malaria endemic regions. Hence, new drugs against *Plasmodium* are urgently needed to control malaria. Our group is engaged in the identification and validation of potential new drug targets to kill *P. falciparum*. Among these new targets is the chaperone protein Hsp90 (heat shock protein 90), a key component of the cell stress response and protein folding machinery. We have shown that Hsp90 inhibition is lethal against *P. falciparum* in vitro. Additionally, the chaperone inhibitors were effective against sensitive and drug resistant strains of the parasite. The tested compounds were very active against the parasites with IC$_{50}$ values between 10$^{-7}$ to 10$^{-5}$M. Finally, the Hsp90 inhibitors limited *Plasmodium* proliferation in vitro not only alone but also in combination with currently used anti-malarial drugs. Some of the combinations displayed synergistic interactions. In parallel, we have cloned all the four genes coding for Hsp90 family members from *P. falciparum* and generated constructs to express the protein chaperones in bacteria. The recombinant proteins have been used to assay the inhibitors specificity in biochemical assays, aimed at determine their mechanism of action. The recombinant chaperones were screened against additional compound libraries to identify new compounds with potential anti-plasmodial activity. Our preliminary results, lead us to conclude that the *P. falciparum* Hsp90 chaperones is an appealing new drug target to combat malaria.
Interleukin-15 (IL-15) plays a critical role in innate and adaptive immunity. IL-15 was found induced in a number of food allergen-induced inflammatory disorders including inflammatory bowel diseases. The purpose of the current study is to test the hypothesis that IL-15 may have a critical role in promoting allergic intestinal disorders. Intestinal IL-15 overexpressed mice were generated by using intestinal fatty acid binding promoter (iFABP). iFABP is specifically present in the jejunum; therefore, IL-15 is overexpressed only in the small intestine (jejunum). Further, to test the role of IL-15 in food allergen-induced intestinal inflammation, FABPi-IL-15 transgenic mice as well as control BALB/c mice were sensitized (Day 1 and Day 14) with a peanut antigen (200 µg peanut/1mg Alum) via intraperitoneal injection, followed by an intragastric peanut antigen challenge (100µg/200 µL and 50 µg/200 µL,) or saline challenge (200 µL) for a total of 8 challenges (Day 21, 23, 25, 27, 29, 31, 33, 35). The body temperature and weight loss were recorded after each challenge, and inflammatory cells accumulation, particularly eosinophil, in the jejunum was assessed by immunohistochemistry, using anti-MBP antibody a marker for eosinophilia. As expected, our analysis indicated that IL-15 is overexpressed only in the jejunum. Of note, the baseline average weight of iFABP-IL-15 mice was significantly low compared to the weight of the control BALB/c mice of same age and sex. Further, iFABP-IL-15 mice continued to lose weight compared to their counter part wild type mice following allergen challenge, indicating that overexpressed IL-15 may have affected the food intake due to indigestion or loss of appetite. In addition, peanut-challenged IL-15 overexpressed mice showed significant temperature drops during the first ten minutes of each challenge, which was lower in the saline challenged, transgenic and wild type mice. The histopathological evaluation of jejunum showed a 3-4 fold increase in baseline eosinophil levels (3.127 ± 0.6907/mm²) in iFABP-IL-15 overexpressed mice compare to the wild type mice (1.045 ± 0.5473/mm²). Furthermore, the intestinal eosinophilia further induced 4-5 fold following peanut challenged in iFABP-IL-15 mice (5.469 ± 1.342/mm²) compared to the peanut challenge wild type mice (1.194 ± 0.5781/mm²). Taken together, our data indicates that IL-15 have a critical role in inducing eosinophil mediated intestinal food allergy that needs more detailed investigation on the role of IL-15 by using iFABP-IL-15 transgenic mice.
THE ROLE OF HSP90 IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTE TO AMASTIGOTE DIFFERENTIATION

Nation CS*, Kelly BL**, Pizarro JC*

*Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA.
** Department of Microbiology, Immunology and Parasitology, School of Medicine, LSUHSC, New Orleans, LA

*Leishmania* is a genus of protozoan parasites transmitted by the phlebotomine sand fly that causes an array of disease ranging from cutaneous to visceral leishmaniasis resulting in significant morbidity and mortality. These parasites have a dimorphic life cycle, existing as a promastigote in the sand fly vector and an intracellular amastigote in the human host. While it is known that temperature and pH changes induce this change, the molecular basis of this differentiation is still unknown. To uncover the molecular mechanisms behind this transition, we used Hsp90 inhibitors, which are also capable of inducing the promastigote to amastigote change. In addition to the expected transition we observed a dose-dependent morphological change in response to the inhibitor, a situation not seen when changing the temperature or pH. We hypothesize that the morphological variability induced by the Hsp90 inhibitor is associated with intermediate states in the promastigote to amastigote transition not seen under normal circumstances. We have employed different techniques to characterize these forms such as morphological analysis by microscopy and FACS, and gene expression analysis by qRT-PCR. We have also generated reporter plasmids expressing fluorescent proteins under the control of promastigote and amastigote specific promoters to follow the transition between the two forms in response to the typical heat and pH as well as in the presence of the Hsp90 inhibitor. Finally, proteomic analysis of promastigotes, amastigotes and intermediate forms will help us identify the effector proteins responsible for the parasite transition. Ultimately, these signaling molecules will represent potential drug targets against leishmaniasis, a neglected disease that badly needs new therapeutic options.
IN GILT-KO MICE, DISULFIDE BOND DELETIONS IN HIV-1 GP120 ALLOW THE PRESENTATION OF CD4+ T-CELL EPITOPIES AROUND A DISULFIDE-BONDED CORE

Nguyen H*, Li T, Steede NK*, Hardin K, Robinson JE**, and Landry SJ*,

*Department of Biochemistry, Tulane University School of Medicine, New Orleans, Louisiana

**Department of Pediatrics, Tulane University School of Medicine, New Orleans, Louisiana

CD4+ helper T cells specific for human immunodeficiency virus type 1 (HIV-1) are associated with control of viremia. Nevertheless, vaccines have had limited effectiveness thus far, in part because sequence variability and other structural features of the HIV envelope glycoprotein (gp120) deflect the immune response. It is suggested a broad CD4+ T-cell response is associated with low viremia, but most individuals generate a narrow CD4+ T-cell response, dominated by a few epitopes. Previous studies indicated that CD4+ T-cell epitope dominance is controlled by antigen three-dimensional structure through its influence on antigen processing and presentation. There are numerous disulfide bonds in HIV-1 gp120 that have the ability to modulate its MHC class II processing and presentation. Three gp120 (strain JR-FL) variants were made, in which a single outer domain disulfide bond was deleted to presumably introduce a local region of flexibility that would locally increase the CD4+ response. Immunization of BALB/c mice with one of these variants resulted in a globally low CD4+ response. It was assumed that the increased instability caused by the disulfide-bond deletion led to the catastrophic degradation of potential epitopes. Decreasing the reduction of disulfide bonds in the endosome could prevent this loss of epitopes and instead allow a local increase in the CD4+ response due to a single disulfide-bond deletion. Gamma interferon-inducible lysosomal thioreductase (GILT) catalyzes the reduction of disulfide bonds in the endosome. In this study, GILT-KO mice were immunized with the three disulfide-bond deletion variants. In this GILT -/- context, either single disulfide-bond deletion increased the immunogenicity of epitopes around a disulfide-bonded core, centered where the N-terminus of a conserved, solvent-inaccessible epitope and two other epitopes meet.
ENDOMORPHIN ANALOG ANALGESICS LACK REINFORCEMENT QUALITIES AND ARE PROMISING CANDIDATE MEDICATIONS FOR THE TREATMENT OF OPIOID ADDICTION

Nilges MR*, Cable C*, Zhang X**, Zadina JE***
*Neuroscience Program, **Departments of Medicine and ***Pharmacology, Tulane University, Tulane University School of Medicine, New Orleans, LA

Endomorphins (EM) have the highest selectivity of any endogenous peptide for the pain modulating mu-opioid (MOR) receptor. We have synthesized several metabolically stable, blood-brain barrier permeable EM analogs that provide equal or greater analgesic potency than morphine, the current gold standard MOR agonist. Morphine and other exogenously derived opioid analgesics produce detrimental side effects such as respiratory depression, tolerance, glial cell activation, and have high potential for abuse. We have shown that EM analogs do not induce respiratory depression, produced substantially less tolerance than morphine, and do not induce markers of microglia or astrocyte activation. In abuse liability assays, morphine produced a robust and dose-dependent conditioned place preference effect, but the analogs did not. In self-administration models lever pressings for morphine progressively escalated as lever pressing requirements increased or the available mg/kg dose decreased, suggesting high abuse liability. By contrast, rats with access to intravenous (i.v.) EM analog infusions lever pressed no more than controls with access to i.v. vehicle, suggesting the analogs lack reinforcing qualities. We then trained rats to discriminate i.v. infusions of morphine from vehicle in a 2-lever food choice drug-discrimination (DD) task to assess the substitution potential of the analogs for morphine. Methadone fully substitutes for morphine, but suppresses motor/appetitive responses for food in the DD model. A compound which lacks self-administrations but substitutes for morphine without motor/appetitive impairment has potential as a pharmacotherapy for opioid addiction. We found that the analogs fully substituted for morphine, but did not impair motor/appetitive responding, suggesting the analogs have a more favorable substitution profile than methadone. These data indicate EM analogs have a better safety profile than morphine, lack reinforcement qualities, provide potent analgesia, and are novel candidates for opioid addiction pharmacotherapy.

This work was supported by the Louisiana Board of Regents, the Department of Veterans Affairs, the Office of Naval Research, and the Department of Defense.
SELECTIVE MEMBRANE EXPRESSION OF DAD1 IS ASSOCIATED WITH SURVIVAL OF PROSTATE CANCER CELLS


*Department of Urology, Tulane University School of Medicine, New Orleans, Louisiana
**Department of Pathology, Tulane University School of Medicine, New Orleans, Louisiana

Defender against Apoptotic Cell Death is negative regulator of programmed cell death it was initially identified in the temperature sensitive tsBN7 cell line. It has been shown to be an essential subunit of oligosaccharyltransferase. The latter transfers GlcNAc2-Man9-Glc3 en bloc from dolichol pyrophosphate to specific asparagine residues on the nascent polypeptide chain in the Endoplasmic reticulum. OST catalyzed glycosylation, must be located within a triplet sequence of the Asn-X-Thr-Ser-Cystype, where X can be any amino acid except proline. DAD1 has been found to be mutated in the cancer of prostate, ovary, large intestine and endometrium. Although mechanisms are poorly understood, mutation (SNP) and up-regulation of DAD1 has been associated with sporadic neuroendocrine tumor risk and ovarian cancer therapeutic resistance, respectively. Based on the aforementioned evidence our study attempts to unravel cellular localization and underlying mechanisms by which DAD1 mediates prostate cancer cell survival.

PC-3, RWPE1, primary prostate epithelial cells (PrEC) were obtained from ATCC and the C4-2B cells were generously provide by Dr. L. Chung’s lab. C4-2B and PC-3 were grown in RPMI supplemented with 10% FBS and 1% Penicillin/ Streptomycin. PrEC and RWPE1 cells were grown in special media recommended by the company. Using standard protocols, immunofluorescence with DAD1 antibody (Santa Cruz) was performed in 8-well chamber slides, whereas Immunohistochemistry was performed on tumor sections obtained from LCRC Bio-specimen Core. Plasma membrane proteins were isolated using the cell surface protein isolation kit from Peirce as per manufacturer’s guidelines. Selective expression of DAD1 on prostate cancer cells was determined by LC-MS/MS analysis. Immunoblot analysis was performed using reagents from Odyssey according to a standard protocol. Cell viability assay (72hr) was performed by treating the cells with a neutralizing antibody against DAD1 using WST-8 (Sigma). Percent cells toxicity following 72 hour treatment with neutralizing antibody was determined by LDH assay kit from Roche. Unlike normal cells, cancer cells show increased expression of DAD1, particularly on the plasma membrane, as evidenced by LC-MS/MS, immunocytochemistry, immunohistochemistry and immunoblot assays. DAD1 neutralizing antibody shows up to 60% cell death in prostate cancer cells and 70% cell toxicity compared to the normal IgG treated controls. In contrast, primary prostate epithelial cells showed no significant cell death or toxicity when treated with DAD1 antibody compared to the normal IgG treated controls. Our data suggests that localization of DAD1 to the plasma membrane is crucial to the survival of prostate cancer cells. Since the role DAD1 has not been explored in cancer further studies are required to unravel the underlying mechanisms and to explore its potential in as a target in prostate cancer therapy.
More than 200 million US gallons of light crude and 1.8 million US gallons of dispersants were released into the Gulf of Mexico during the Deepwater Horizon oil spill. The National Oceanic and Atmospheric Administration (NOAA), in conjunction with United States Federal Food and Drug Administration (FDA), closed an estimated 88,500 square miles of federal waters to fish and oysters harvesting in order to safeguard the safety of gulf seafood among high end consumers, especially vulnerable populations of pregnant women, children and elderly. Consequently, a comprehensive re-opening protocol was developed to outline the criteria for the re-opening of harvest areas, depending on the outcome of sensory and chemical analysis of the seafood samples collected from the closed federal and state harvest waters. In addition, health risk assessment was conducted by the FDA with the calculation of the Levels of Concern (LOCs) that represent the benchmarks of safety from the presence of potential contaminants and toxicants from seafood consumption. In this study, we evaluated the impact of the disaster and response on the perception of seafood safety among consumers in the affected study population. Our primary aim involved; analysis of the health risk assessment and its accuracy in the estimation of the overall health risks and potential hazards associated with the disaster. The secondary aim entailed; determination of reference standards (based on the results of the health risks analysis) and recommendation of options for improvement with respect to health risk parameters among vulnerable populations in future oil spill disaster and response.
TRPV1 IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IS INVOLVED IN THE REGULATION OF GLUCOSE HOMEOSTASIS

Miyata K**, O'Hare JD**, Fourrier TL**, Krantz AM**, Zsombok A*, **, ***

*Department of Physiology, **Neuroscience Program, ***Department of Medicine, Endocrinology Section, Tulane University, New Orleans, LA 70112
*Equal contribution

The autonomic nervous system plays a pivotal role in the regulation of glucose homeostasis. Transient receptor potential vanilloid type 1 (TRPV1) receptors are expressed in the paraventricular nucleus (PVN) of the hypothalamus and liver-related PVN neurons are controlled by TRPV1-dependent excitatory neurotransmitter release. However, the effect of TRPV1 activation on whole body glucose homeostasis remains uncertain. Capsaicin, a TRPV1 agonist, was injected bilaterally into the PVN of mice while systemic blood glucose levels, serum insulin, and serum glucagon levels were measured. Expression of proteins involved in hepatic gluconeogenesis and in skeletal muscle glucose uptake was also measured. Activation of TRPV1 in the PVN significantly decreased systemic blood glucose levels. The glucose lowering effect of TRPV1 was suppressed in the presence of the glutamate receptor antagonist, kynurenic acid, indicating that TRPV1 exerts its effects through excitatory neurotransmission. Serum insulin levels were not different between groups. Activation of TRPV1 in the PVN decreased hepatic PEPCK and pyruvate carboxylase expression. In skeletal muscle, phosphorylation of glycogen synthase was significantly decreased. Taken together, our data demonstrate that TRPV1 in the PVN modulates systemic glucose homeostasis.

This work was supported by Tulane Aging COBRE (P20GM103629), COBRE in Hypertension (P30GM103337), Tulane Neuroscience Bridge Grant and Tulane School of Medicine Pilot Project award.
DISPARITIES IN 30-DAY READMISSIONS AFTER TOTAL HIP ARTHROPLASTY

Oronce CIA*, Shao H**, Shi L**

*School of Medicine, Tulane University, New Orleans, LA
**Department of Global Health Systems and Development, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA

The Centers for Medicare and Medicaid Services (CMS) levies penalties on hospitals with higher than expected thirty-day readmission rates for specific conditions and will expand this penalty in fiscal year 2015 to include total hip arthroplasty (THA). While THAs have relatively lower readmission rates compared to other common procedures, CMS has an interest in reducing their frequency because of their volume and aggregate cost. Previous work has identified racial disparities in readmission rates for myocardial infarction, heart failure, and pneumonia, however, little is known of disparities in THA readmissions across the general population. Our objective was to identify disparities in THA readmissions based on race and type of insurance. We conducted a retrospective cohort study using the California State Inpatient Database from the Healthcare Cost and Utilization Project of the Agency of Healthcare Research and Quality to identify index hospitalizations for primary THA and calculated 30-day all-cause readmission rates for specific demographics and types of insurance. We constructed four multivariate logistic regression models with varying levels of risk-adjustment to predict the odds of readmission based on race and insurance type. Our sample consisted of 50,122 patients discharged from California hospitals from 2009 through 2011 following a total hip arthroplasty. The overall rate of 30-day all-cause readmissions was 4.6 percent. African-American patients had a higher risk of readmission than white patients after THA (adjusted odds ratio [aOR], 1.53; 95 percent confidence interval [CI], 1.29-1.82), as did Hispanic patients (aOR, 1.33; 95 percent CI, 1.15-1.56). Patients covered under Medicaid (Medi-Cal) also experienced higher risk of readmission compared to those not covered under Medicaid (aOR, 2.00; 95 percent CI, 1.60-2.50). In summary, we found significant differences in the odds of thirty-day readmissions for African-American, Hispanic, and Medicaid patients in the state of California after a total hip arthroplasty. Private payers often follow CMS’s lead on reimbursement policies and thus CMS readmission penalties will have a serious impact on hospital finances. As hospitals accelerate efforts to reduce readmissions, payers should keep mind of how readmission penalties may impact hospitals that care for vulnerable patient populations.

This work was supported by the endowed Regents Professorship of the Tulane University School of Public Health and Tropical Medicine.
Autophagy inhibitors enhance ribavirin antiviral activity against HCV in cell culture

Rajesh Panigrahi\textsuperscript{1*}, Partha K Chandra\textsuperscript{1}, Pauline Ferraris\textsuperscript{1}, Ramazan Kurt\textsuperscript{1,2} and Srikanta Dash\textsuperscript{1,2}

\textsuperscript{1}Pathology and Laboratory Medicine, \textsuperscript{2}Department of Medicine, Gastroenterology and Hepatology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA-70112.

Research Program: Immunology, Infection & Inflammation

Introduction: Ribavirin (RBV) remains an important component of interferon free regimen but the antiviral mechanism of RBV against HCV is not understood well. We reported that RBV inhibits HCV replication by blocking at the level of internal ribosome entry site (IRES) mediated translation; however, the antiviral activity of RBV is impaired in persistently HCV infected culture.

Aim: To investigate the mechanisms of impaired antiviral activity of RBV using persistently infected HCV cell culture model.

Methods: Persistently infected HCV cell culture model was developed in Huh 7.5 where viral replication was measured by Renilla-luciferase reporter assay and immunohistochemical staining of HCV core protein. Long-term antiviral activity of RBV in HCV cell culture was studied by the measurement of Renilla-luciferase, immunohistochemical staining of core protein and RBV uptake assays using \[^{3}\text{H}]\) labeled RBV. The effect of HCV induced autophagy response on the expression of RBV transporters and RBV uptake was compared.

Results: We established a persistently infected HCV cell culture model in Huh 7.5 cells that support HCV replication for an extended period. We found that RBV (20 µg/mL) antiviral activity was strong at the early-infected culture, which was progressively impaired in the persistently infected culture with a maximum 20% inhibition at day 11, whereas a 90% inhibition of HCV replication was observed with IFN-\(\lambda\) treatment (25 ng/mL). Our results show that HCV infection induces autophagy response confirmed by decrease in p62 expression and increased expression of Beclin 1. We provide evidence for the first time that autophagy response due to HCV replication in the persistently infected culture impairs the expression of ENT1 required for RBV uptake and antiviral activity. Autophagy induction by known chemical (Torin1) showed a down regulation of RBV transporters and significant decrease in RBV uptake. Furthermore, inhibition of autophagy by hydroxychloroquine (HCQ) or 3-Methyladenine (3-MA) rescues the RBV transporters and significantly enhances the antiviral activity of RBV.

Conclusions: Our results indicate that autophagy response due to HCV replication in the persistently infected culture degrades ENT1 that impairs RBV uptake and antiviral activity. We proposed that inhibition of autophagy process is an alternative strategy to improve the antiviral activity of RBV against HCV infection.
BURKHOLDERIA PSEUDOMALLEI OUTER MEMBRANE VESICLE VACCINE PROVIDES SIGNIFICANT PROTECTION AGAINST SEPTICEMIC INFECTION WITH A HETEROLOGOUS STRAIN


*Tulane Univ., New Orleans, LA, **Univ. of Texas Med Branch, Galveston, TX, ***Tulane Natl. Primate Res. Ctr., Covington LA.

The environmental, Gram-negative, encapsulated bacillus, Burkholderia pseudomallei, is the causative agent of melioidosis, a disease associated with high morbidity and mortality in endemic areas of Southeast Asia and Northern Australia. B. pseudomallei is also classified as a Tier I select agent due to the bacterium’s high lethality and innate resistance to antibiotics as well as the lack of an effective vaccine against this organism. Gram-negative bacteria, including B. pseudomallei, secrete outer membrane vesicles (OMVs) which are enriched with multiple protein, lipid, and polysaccharide antigens. Previously, we demonstrated that immunization with multivalent B. pseudomallei-derived OMVs protects highly susceptible BALB/c mice against lethal aerosol challenge. In addition to inhalational exposure, percutaneous inoculation with one of numerous diverse environmental B. pseudomallei strains poses a significant concern and challenge to vaccine prevention. In this work, we evaluated the protective efficacy of OMV immunization against septicemic infection with a heterologous strain. We demonstrate that B. pseudomallei OMVs derived from strain 1026b afford significant protection against systemic challenge with B. pseudomallei strain K96243 up to 21 days post-infection. OMV immunization delayed disease progression and reduced early splenic bacterial burdens. OMV immunization induced robust OMV-, LPS-, and CPS-specific serum IgG (IgG1, IgG2a, and IgG3) and IgM antibody responses. OMV-immune sera promoted bacterial killing in vitro, and passive transfer of OMV-immune sera protected naïve mice against subsequent challenge. These results indicate that OMV immunization provides antibody-mediated protection against acute, rapidly lethal sepsis in mice. B. pseudomallei-derived OMVs may represent an efficacious multivalent vaccine strategy against melioidosis.
Genome-wide DNA Methylation Network Analysis for Osteoporosis Risk

Chuan Qiu¹, Hui Shen¹, Jian Li¹, Hong-Wen Deng¹,²

1. Center for Bioinformatics and Genomics, Department of Biostatistics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA
2. Center of Systematic Biomedical Research, University of Shanghai for Science and Technology, Shanghai 200093, P. R. China

Background: Osteoporosis is a common disease mainly characterized by low bone mineral density (BMD) and increased risk of fractures. Peripheral blood monocytes (PBMs) may act as precursors of osteoclasts, the bone resorption cells, and also produce cytokines important for osteoclast activity, and thus represent major systemic target cells for bone metabolism. Alterations in DNA methylation has been implicated as a key regulatory mechanism in the etiology of human complex diseases. Recent studies suggested that DNA methylome is organized into modules of co-methylated features. In this study, we carried out a network analysis to construct modules of highly co-methylated gene promoters in PBMs and identify modules that are significantly associated with BMD.

Methods: Genome-wide DNA methylation profiles were generated by MeDIP-seq in PBMs from 18 unrelated Caucasian postmenopausal females with extremely high (n=9) and low (n=9) hip BMDs. MeDIP-seq signals were normalized and quantified using the MEDIPS analysis package. By focusing on the promoter DNA methylation data, we applied a weighted correlation network analysis (WGCNA) to identify the co-methylation modules and summarize the methylation profiles of each module into a single representative eigengene value. The eigengene values of individual modules were compared between the high and low BMD groups to identify co-methylation modules associated with BMD.

Results: We identified a total of 18 co-methylation modules, each ranging in size of 32-251 gene promoters. Specifically, the overall methylation level of module-18 was significantly higher in the low-BMD group (p=0.002). Gene ontology analysis suggested that module-18 was highly enriched for genes belonging to a number of interesting biological processes, such as “cellular response to vitamin D” (p=1.75E-16), “blood vessel endothelial cell migration” (p=4.33E-12), and “cellular response to mechanical stimulus” (p=2.07E-09). Interestingly, several of the module-18 genes (e.g., AQP9, ITGB1) have been associated with BMD variation through previous genome-wide association studies.

Conclusions: Using system level network analysis, we reconstructed the promoter co-methylation network in PBMs and identified a co-methylation module that may mediate variation in risk to osteoporosis. Our results highlighted the advantages of using systems-level network analysis to add value to the traditional DNA methylation analysis.
STUDY OF REGENERATIVE PROCESSES USING *IN VITRO* MODEL SYSTEMS

Quijano LM, Lynch KM, Ahsan T.

Department of Biomedical Engineering, Tulane University, New Orleans, LA.

The ability to induce limb regeneration in humans is of growing interest in the field of regenerative medicine. Unlike amphibians, which can re-establish complex structures after amputation, mammals have limited regenerative capabilities. In work done by the group of Ken Muneoka (Tulane), *in vivo* mammalian epimorphic regeneration has been in the mouse digit tip, which was found to present a level-specific response after amputation, where the terminal phalangeal element (P3) results in regeneration but not the next more proximal joint (P2). Previously, our laboratory characterized P2 and P3 cells (a generous gift from Dr. Ken Muneoka) migration and proliferation patterns *in vitro*, two critical processes in epimorphic regeneration. Cells from the regenerative P3 element had markedly higher proliferative rates *in vitro* compared to similar cells from the non-regenerative P2 element, both in fibronectin coated tissue plastic (Fn-TC) and in suspension cultures (SUS).

After an injury, a potential regenerative process may be dependent on microenvironmental changes, such as changes in oxygen concentration and interactions with adjacent cells and growth factors. The objective of this study was to evaluate the effect of the changes in the physicochemical microenvironment on P2 and P3 proliferation by switching the oxygen concentration and by evaluating the effects of trophic factors of adjacent cells.

P2 and P3 cells were cultured in Fn-TC and exposed to distinct oxygen levels for both a primary treatment of 2.5 days and then passaged for a secondary treatment of 2 days (maintained at or switched between low (1%) and high (21%) concentration). Cell number and cell cycle inhibitors were evaluated. P3 cells were regulated by oxygen level, while P2 cells were primarily regulated by a switch in oxygen concentration. Of the inhibitors tested, only p15 was also regulated by a switch in oxygen for P2 cells.

To evaluate the effects of trophic factors of adjacent cells, P2 and P3 cells were co-cultured with fibroblasts and Mesenchymal Stem Cells (MSC). P2 and P3 cells were more responsive to fibroblasts than MSCs in regards to proliferation and migration. Exogenous BMP added to the media induced the same response in P2 and P3 cells when cultured with fibroblasts. MSC were not found to play a major role in P2 and P3 cell proliferation and migration.

Through the use of different microenvironments we hope to identify specific mechanisms that control regenerative cellular processes. Future work may explore deeply the effects of fibroblasts and MSC paracrine signaling on P2 and P3 cells (independently and together), to evaluate changes in differentiation induction, spatial patterning and eventual 3D structure formation.
MEMBRANE GLUCOCORTICOID RECEPTOR ACTIVATION INITIATES RAPID NUCLEAR LOCALIZATION OF CYTOSOLIC GLUCOCORTICOID RECEPTOR IN HYPOTHALAMIC NEURONS


*Department of Cell & Molecular Biology, Tulane University, New Orleans, LA
**Neuroscience Program, Tulane University, New Orleans, LA

Stress activates signaling through the HPA-axis, which involves negative feedback from adrenal glucocorticoids through receptors in the hypothalamus. Classic glucocorticoid receptor (GR) signaling involves ligand binding, release from chaperone proteins in the cytosol, and translocation into the nucleus where GR dimerizes and acts as a transcription factor to modulate genes downstream of glucocorticoid response elements. Treatment of cells with ligand, such as the synthetic cortisol dexamethasone (Dex), rapidly induces nuclear localization of GR. Although the mechanism of this translocation is not known, there is evidence that ligand binding and participation of the chaperone complex are necessary for entry into the nucleus. However, recent data from a murine hypothalamic cell line (mHypoE-N11), that endogenously expresses GR, demonstrate that Dex conjugated to bovine serum albumin (Dex-BSA) is also able to rapidly induce translocation of the cytoplasmic GR (cGR). Dex-BSA is limited to the plasma membrane by BSA, which is too large and hydrophilic to cross the lipid layers of the membrane. Intensity analysis of the nuclear to cytoplasmic ratio of GR-immunoreactivity is increased by both Dex and Dex-BSA treatment within 10 minutes of treatment, and persists for at least 3 hours. The data suggest that a membrane glucocorticoid receptor (mGR) exists on the cell surface that is able to bind Dex-BSA and relay an intracellular signal that results in translocation of cGR to the nucleus in its unliganded form. Preliminary ICC and Western blotting of subcellular fractions with GR antibody shows possible membrane localization of the receptor in N11. MAPK, PKA, and PKC activators and inhibitors were used to identify possible roles of kinase pathways in mGR initiated cGR nuclear localization. Further investigation into alternate pathways, or phosphorylation studies may help elucidate the details of this process.

This work was supported by Tulane Start-up Funds to NV.
RECOMBINANT PFS25 PRODUCED IN *E. coli* INDUCES HIGHLY POTENT *PLASMODIUM FALCIPARUM* TRANSMISSION BLOCKING IMMUNITY

Kumar Rajesh*, Angov Evelina**, Kumar Nirbhay*

Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street (SL-17), New Orleans, LA 70112*, Walter Reed Army Institute for Research, Silver Spring, MD 20910 **.

Pfs25 expressed on the surface of zygotes and ookinetes, is a well-established malaria transmission blocking vaccine target antigen. Production of Pfs25 in native functional conformation with potential to elicit effective immunogenicity without need for any further chemical conjugation still remains a major challenge. In the current study, codon harmonized recombinant Pfs25 (CH-rPfs25) was expressed in *E. coli* in monomeric form retaining reduction-sensitive conformational epitopes of transmission blocking monoclonal antibodies. CH-rPfs25 formulated in several adjuvants elicited strong immunogenicity in pre-clinical studies in mice. Immunization induced high titer of antibodies recognizing native Pfs25 on the surface of live gametes of *Plasmodium falciparum* in immunofluorescence assays, and the antibodies demonstrated complete transmission blocking activity in mosquito membrane feeding assays. The transmission blocking efficacy was dose dependent - 100% blocking even after 1:128 dilution of sera from mice immunized in complete Freund’s adjuvant or Montanide ISA51 and 1:16 dilution of sera from the Alum group. The blocking was mediated by antibodies and concentration as low as 31.25 µg/ml of total purified IgG exhibited 100% transmission blocking in *Anopheles gambiae* and *An. stephensi* mosquitoes. This study provides first ever evidence for successful expression and purification of functional rPfs25 in *E. coli* with extremely potent transmission blocking activity. Our results suggest that CH-rPfs25 expressed in *E. coli* can be further developed into vaccine formulations for evaluation in larger animals including human clinical trials. An effective transmission-blocking vaccine will play a central role in the goal of gradual malaria elimination and eventual eradication.
A CAUSAL ROLE FOR INFLAMMATION IN THE PATHOGENESIS OF LYME NEUROBORRELIOSIS


*Division of Bacteriology and Parasitology, Tulane National Primate Research Center, Covington, LA, **Division of Comparative Pathology, Tulane National Primate Research Center, Covington, LA, ***Division of Veterinary Medicine, Tulane National Primate Research Center, Covington, LA, **** Department of Neurology, Louisiana State University Health Sciences Center, New Orleans, LA, and *****Department of Microbiology and Immunology, Tulane University Medical School, New Orleans, LA

Lyme neuroborreliosis (LNB), caused by the spirochete Borrelia burgdorferi (Bb) affects both the peripheral and the central nervous systems. Radiculitis or spinal nerve root inflammation, which can cause pain, sensory loss, and weakness, is the most common manifestation of peripheral LNB in humans. We previously reported that rhesus monkeys infected with Bb develop radiculitis and inflammation in the dorsal root ganglia (DRG), with elevated levels of neuronal and satellite glial cell apoptosis in the DRG. We hypothesized that Bb induces the production of inflammatory mediators in glial and neuronal cells that promote the acute cellular infiltration of immune cells into the nervous system, and that this inflammation has a role in potentiating glial and neuronal apoptosis. A causal role for inflammation in mediating LNB was assessed by evaluating the inflammatory changes induced in the central nervous system (CNS), spinal nerves and ganglia (DRG) of rhesus macaques that were inoculated with live Bb into the cisterna magna, and treated in parallel with either dexamethasone, a steroidal anti-inflammatory drug, or meloxicam, a non-steroidal COX-2 inhibitor. At necropsy, we characterized the lesions for inflammatory cells, presence of Bb antigen, neurodegeneration and demyelination, and evaluated apoptosis in DRG cells. Inflammatory mediator ELISA of the cerebrospinal fluid (CSF) showed significantly elevated IL-6, IL-8, CCL2, and CXCL13 as early as one week post-inoculation, accompanied by lymphocytic and monocytic pleocytosis in all of the infected animals, except those that were treated with dexamethasone. CSF cell pellets and CNS tissues of infected animals were culture-positive for Bb. Histopathology revealed multifocal leptomeningitis, vasculitis, radiculitis, and DRG inflammatory lesions in infected animals that were left untreated or treated with meloxicam, but not in dexamethasone-treated animals. Neurodegeneration and focal myelitis was observed in the cervical spinal cord. Neuritis in the DRG and spinal cord, and demyelination in the dorsal roots were confirmed by Fluoro Jade-C and Luxol Fast-Blue staining, respectively. Confocal microscopic evaluation revealed Bb antigen in the DRG and dorsal roots. Importantly, the DRG of all infected animals except those that were treated with dexamethasone showed significant neuronal and satellite glial cell apoptosis in addition to apoptosis in the dorsal roots, by the activated caspase-3 and TUNEL assays. Our results show that neuronal and glial apoptosis in the DRG occurs in the context of inflammation induced by the Lyme-disease spirochete, suggesting that inflammation has a causal role in the pathogenesis of acute LNB.

This project was supported by the National Institute of Neurologic Disorders and Stroke through grant NS048952, and by the Office of Research Infrastructure Programs (ORIP) of the National Institutes of Health through grant P51OD011104.
STEM CELL-BASED SELECTIVE DELIVERY OF ALPHA KETO REDUCTASES FOR THERAPEUTIC TARGETING OF RESIDUAL ANDROGENS IN METASTATIC PROSTATE CANCER

Ranjan Manish1*, Naga M Kommu1, Zakaria Abd Elmageed1, Steven E. Braun2,4, Debasis Mondal2,3 and Asim B. Abdel Mageed1,2,3. Tumor Biology and Signaling, Departments of Urology1 and Pharmacology2 and Tulane Cancer Center3, School of Medicine, and Tulane National Primate Research Center4, Tulane University, 1430 Tulane Avenue, New Orleans, LA-70112.

Background: Androgen-deprivation therapy (ADT) is the mainstay treatment for patients with metastatic prostate Cancer (PC). Although initially effective, hormonal therapy is marked by progression to castration-resistant PC (CRPC) over a period of 18–20 months, with median survival of 1–2 years. Importantly, large body of evidence indicate that in the setting of ‘castrate’ serum testosterone levels, prostatic androgen concentrations remain at approximately 10–25% of the levels found in untreated patients. The latter is well within the range capable of sustaining androgen-receptor (AR) signaling and tumor growth. This study explores if selective delivery and inactivation of “intracrine” androgens by alpha keto reductase-expressing adipose stem cells (ASCs) inhibit prostate tumor clonal expansion in metastatic microenvironments. Our long-term goal is to establish a multi-modality hormone targeting therapy to reduce tumor burden in patients, especially among African American men.

Methods: The Akr1c14 (NM_138547) rat cDNA clone coding for 3α-hydroxysteroid dehydrogenase (3α-HSD), also known as aldo-keto reductase family 1 member C4 (AKR1C4), was obtained from Origene. The IL2-SS (signal sequence) was synthesized (IDT, Inc.) to enable secretion of Akr1C14 gene production by the recipient cells. The 3α-HSD-IL2-SS was subcloned in a bicistronic lentivirus expression vector, pLVX-IRES-ZsGreen1 (Clontech, Inc.). Initially, the Akr1c14 gene was cloned in-frame with IL-2SS at the N-terminus in pCR-II plasmid using EcoRI and EcoRV restriction enzymes and adaptors and the DNA insert was sequence verified. The IL-2SS-Akr1c14 sequence was PCR amplified with SpeI and NotI anchored primers. The PCR product was subcloned in SpeI-NotI digested pLVX-IRES-ZsGreen1 plasmid to generate pLVX-IL-2SS-Akr1c14-IRES-GFP construct. Virus production was verified with Lenti-X GoStix™. Transgene production was monitored in PC cells and ASCs by immunoblot analysis. PC cell viability was assessed by WST-8 assay. Testosterone & PSA levels were monitored using ELISA (Cayman) and Dual-Luc assay (Promega), respectively.

Results: The titers for EF1-alpha GFP and IL-2SS- Akr1c4-GFP lentiviruses were 2.61 ± 0.29 x 10^8 and 3.26 ± 0.53 x 10^8, respectively. The 3α-HSD was efficiently expressed and produced by transduced cells, as evidenced by immunoblot assays. The rh3α-HSD was able to reduce DHT levels by ~ 50%, as measured by ELISA. The growth and viability of hormone-dependent PC cells (LNCaP) was reduced by 40% in AKR1C14 transfected cells compared to DHT treated cells in vitro. Growth inhibition was associated with 40% reduction in PSA expression level in LNCaP cells treated with AKR1C14 conditioned media compared to controls. The inhibition of growth and PSA expression was attributed to reduction (26.30%) in secreted testosterone level in cells treated with AKR1C14 conditioned media compared to controls.

Conclusion: Our data demonstrates that selective delivery of alpha keto-reductases by allogeneic stem cells could effectively hydrolyze residual androgens and inhibit AR transactivation and prostate cancer cell growth in vitro. Further in vivo studies are required to validate its therapeutic potential on metastatic PC under ADT.
NEW THERAPEUTIC STRATEGIES TO PREVENT CONTRAST-INDUCED NEPHROPATHY USING HUMAN KIDNEY PROXIMAL TUBULE EPITHELIAL CELLS

Rathmell K*, Khan AM*, Maderdrut JL**, Simon EE***, and Batuman V***

*Section of Nephrology & Hypertension, **Peptide Research Laboratory, Department of Medicine, Tulane University, School of Medicine, New Orleans, LA; and ***Veterans Affairs, Southeast Louisiana Veterans Health Care System, New Orleans, LA.

Purpose: Contrast-induced nephropathy (CIN) is defined as an acute decline in renal function following the intravascular administration of contrast media (CM) in the absence of other causes. Older patients with diabetes and chronic kidney disease undergoing radiocontrast procedures are at increased risk of CIN with a rate approximately 4-fold higher than those without diabetes or preexisting renal impairment. Renoprotective effects of pituitary adenylate cyclase-activating polypeptide 38 (PACAP38) and N-acetyl-L-cysteine (NAC) have been shown in various models of acute kidney injury. However, effects of PACAP38 and NAC, together, on modulating inflammatory pathways activated by CIN have not been studied. We hypothesize that hyperglycemic human kidney epithelial (HK-2) cells are more vulnerable to injury than normoglycemic HK-2 cells and combination therapy with PACAP38 and NAC is more effective in preventing CM-induced injury to HK-2 cells than either agent alone.

Methods: To determine the optimal dose of CM for in vitro cytotoxicity, HK-2 cells were exposed to ionic (Urografin) or nonionic (iohexol or iodixanol) CM at a dose range of 25–100 mg iodine/ml for 6–24 hr. Before exposure to CM, hyperglycemia was induced in HK-2 cells with 25 mM glucose or mannitol (osmolal control) for 6–24 hr. One hr prior to CM exposure, cells were pretreated with PACAP38 in a dose range of 10^{-9} M-10^{-6} M or with NAC in a dose range of 0.5 mM-10 mM. Also combinations of PACAP38 and NAC at several concentrations were tested. The renoprotective effects of PACAP38 and NAC were evaluated using lactate dehydrogenase activity and cell proliferation assays.

Results: Both ionic and nonionic CM caused significant cytotoxicity at 50 mg iodine/ml in HK-2 cells and Urografin was the most toxic followed by iohexol and then iodixanol. Cells treated with glucose or mannitol combined with iodixanol showed a significant (p < 0.01) decrease in cell proliferation compared to cells treated only with iodixanol. There was no significant difference among cells treated with glucose or mannitol or iodixanol alone, although both treated cells were significantly (p < 0.001) different from untreated cells. There was also no significant difference among osmolalities of glucose (25 mM), mannitol (25 mM), iodixanol (50 mM), and 25 mM glucose + 50 mM iodixanol measured independently in cell culture medium (mean osmolality, 312.5 ± 15.7 S.D. mOsm/kg). PACAP38 (10^{-6} M) and NAC (2 mM) significantly (p < 0.01) restored cell proliferation inhibited by iodixanol. A lower dose combination of PACAP38 (10^{-7} M) and NAC (0.5 mM) was more effective in preventing injury than either of these agents alone.

Conclusions: The isosmolar nonionic CM iodixanol exerted toxicity on HK-2 cells and hyperglycemic HK-2 cells exhibited more contrast-induced toxicity than normoglycemic HK-2 cells. Combination therapy with PACAP38 and NAC was more effective than treatment with either of these agents alone and could be used as a new therapeutic regimen in further studies using animal models.

This work was supported by a research grant from NKFL.
Title: Expanded newborn screening in Louisiana 2006-2013: Results and Outcomes

Authors: RM Reimers, P Floyd-Browning, DW Baye, T Crockett, C Harris, E Morava, M Marble, HC Andersson.

Background: Tandem mass spectrometry (MS/MS) has been used for nearly a decade for expanded newborn screening (eNBS) of inborn errors of metabolism. The Louisiana eNBS pilot program began in November, 2004. After Hurricane Katrina, from August, 2005 to August, 2007 all screens were performed at Iowa State Hygienic Laboratory until the Louisiana state lab reopened state in 2007. This paper describes the incidence of 27 screened diseases from June 2006 to June 2013.

Methods: Information was abstracted from the state screen tracking database and corroborated with confirmatory results from the two state treatment centers at Louisiana State University Health Sciences Center and Tulane University. Clinical outcomes were obtained from medical records and verified with the clinicians and dietitians.

Results: 449,234 Infants were screened and 311 true positives confirmed. The overall incidence was 1: 1,444 in seven years, or 1: 10,108 per year. The five most common conditions were DG Galactosemia, Biotinidase Deficiency, MCADD (Medium Chain Acyl-CoA Dehydrogenase Deficiency), PKU (Phenylketonuria)/ Hyperphenylalaninaemia, and SCADD (Short-chain acyl-CoA dehydrogenase deficiency). Of the cases, 66% were Caucasian, 20% Black, and 4% Hispanic while the ethnic distribution of all state births which was 57%, 39%, and 6% respectively. No cases developed severe mental retardation (MR) and eight known deaths occurred. Four infants died before screening results were available, two infants died from non-genetic provider mismanagement at one month and one year, one infant died from severe cranial and cardiac anomalies secondary to maternal PKU, and one cause of death was unknown due to loss to follow up after three months.

Conclusion: MCADD and PKU incidences were similar to a previously reported US cohort in North Carolina, however SCADD was over six times more common in Louisiana. Of true positives, Caucasians were overrepresented and Hispanics and Blacks underrepresented. Since 2006, eNBS has successfully ascertained over 300 infants whose inherited disease would in many cases otherwise have lead to death and/or MR. Early ascertainment and treatment has lead to normal or near normal outcomes.
THE TUMOR SUPPRESSOR LIVER KINASE B1 INHIBITS TRIPLE-NEGATIVE BREAST CANCER CELL METASTASIS VIA REGULATION OF AP-1 SIGNALING


*Department of Medicine, Section of Hematology and Medical Oncology, Tulane University School of Medicine, New Orleans, LA, **Department of Cellular and Integrative Physiology; Obstetrics and Gynecology; Molecular and Cellular Biochemistry Medical Sciences, and ***Center for Genomics and Bioinformatics, Indiana University School of Medicine & Simon Cancer Center, Bloomington, IN

The basal sub-type, which shares features with triple-negative breast cancer (TNBC), is among the most lethal breast cancer subtype, characterized by a highly aggressive and metastatic phenotype. Although pathways that may represent targets for novel therapeutic intervention for basal like breast cancer (BLBC) have begun to be elucidated, the ability to define and selectively target the invasive and metastatic phenotype of basal-type/TNBC remains a major challenge facing the breast cancer field.

Liver kinase B1 (LKB1), also known as serine/threonine kinase 11 (STK11), is a known tumor suppressor in many cancers including breast. Low LKB1 expression has been observed in breast cancer patients, and we report a significant association between loss of LKB1 expression and poor prognosis specifically in the basal sub-type of breast cancer. Induction of LKB1 expression in BLBC cell lines inhibited invasiveness in vitro as well as lung and brain metastatic burden in an orthotopic xenograft tumor model. Further analysis of BLBC cell lines overexpressing LKB1, by next generation sequencing (RNA-seq), revealed striking regulation of metastasis-associated pathways including cell adhesion, extra cellular matrix remodeling, and epithelial-to-mesenchymal transition (EMT). Additionally, LKB1 expression inhibited EMT-associated genes (CDH2, Vimentin, Twist) and induced the epithelial cell marker CDH1, indicating a reversal of the EMT phenotype in a triple-negative breast cancer cell line MDA-MB-231. We further demonstrated marked inhibition of matrix metalloproteinase 1 (MMP-1) expression and activity via regulation AP-1 family member cJun in LKB1 expressing cells. We have demonstrated a role for LKB1 expression in the regulation of cell invasion and metastasis in addition to tumorigenesis. Taken together these data support future development of therapeutic agents to induce the LKB1 signaling pathway in BLBC/triple-negative breast cancer.

This research was supported by: The Department of Defense Breast Cancer Research Program (BC085426); The National Institutes of Health/National Center for Research Resources (P20RR020152 and CA125806).
LACK OF THE PRORENIN RECEPTOR (PRR) IN THE URETERIC BUD (UB) DISRUPTS DEVELOPMENTAL PROGRAMMING OF NEPHROGENESIS

Riedl, Lindsay; Song, Renfang; Yosypiv, Ihor V.
Department of Pediatrics, Hypertension and Renal Center of Excellence
Tulane University School of Medicine, New Orleans, LA

Purpose of Study: Severe reductions in nephron number that are characteristic of renal hypodysplasia (RHD) are the leading cause of childhood end-stage kidney disease. Mice that lack the PRR in the UB lineage (PRRUB−/−) exhibit reduced UB branching, resulting in decreased nephron endowment and RHD (Song. PLoS ONE, 2013). In this study, we tested the hypothesis that decreased nephron endowment in PRRUB−/− mice is due to defects in molecular pathways and cellular mechanisms that control nephrogenesis.

Methods Used: PRRUB−/− (n=3) and control (n=3) mice were studied. Mesenchymal cell proliferation and apoptosis was assessed on kidney sections (3 sections/kidney) using anti-phosphohistone H3 (pH3) and -cleaved caspase 3 (Ca3) antibodies on embryonic (E) days E12.5 and E18.5. Data were normalized to the total number of DAPI-positive cells (Image J). Expression of key genes that control nephrogenesis was studied by real-time qRT-PCR on E12.5. The intensity of Six2 immunostaining (Abcam, 1:200), normalized for the surface area of the kidney section, was quantitated by Slidebook 4.1 software.

Summary of Results: The number of Ca3-positive apoptotic cells was higher (E12.5: 20.7±2.5 vs. 4.0±1.4 p<0.001; E18.5: 10.4±3.8 vs. 2.5±1.2, p<0.05) whereas the number of pH3-positive cells did not differ on E12.5 (56.7±6.8 vs. 61.250±9.674, p=0.5) and was reduced on E18.5 (18.9±2.7 vs. 38.7±3.4, p<0.001) in the mesenchyme of mutant compared with control kidneys. qRT-PCR demonstrated decreased Pax2 (0.64±0.04 vs. 1, p<0.005), Pax8 (0.73±0.01 vs. 1, p<0.001), FGF8 (0.68±0.15 vs. 1, p<0.05), Lhx1 (0.76±0.07 vs. 1, p<0.05) and Wnt4 (0.43±0.2242 vs. 1, p<0.005), and increased Six2 (5.5±0.4 4 vs. 1, p<0.005), Bmp7 (1.44±0.1 vs. 1, p<0.001), Cited1 (1.5±0.23 vs. 1, p<0.05), β-catenin (1.7±0.03 vs. 1, p<0.001), and Wnt9b (13.4±1.1 vs. 1, p<0.001) mRNA levels in mutant compared to control kidneys. Six2 immunostaining was increased in the mutant vs. control kidneys (2664000±309400 vs. 1030000±145200 pixels, p<0.05).

Conclusions: We conclude that lack of the UB PRR disrupts nephrogenesis via: 1) Induction of mesenchymal cell apoptosis, 2) Inhibition of mesenchymal cell survival and 3) Aberrant expression of key genes that direct nephrogenesis. These findings are consistent with the concept that branching UB provides survival and proliferation signals to the cap mesenchyme, which ultimately gives rise to nephrons.
Therapy-Related Mixed Phenotype Acute Leukemia Following RCHOP Chemotherapy for Primary Cutaneous Diffuse Large B-cell Lymphoma

Roberts III EL*, Oncale MB**, Schmieg, JJ*, Sarah HF**

*Department of Pathology and Laboratory Medicine, Tulane University, New Orleans, Louisiana
**Department of Hematology and Medical Oncology, Tulane University, New Orleans, Louisiana

Mixed phenotype acute leukemia is a rare entity that accounts for about 2-5% of all acute leukemias. Therapy-related mixed phenotype acute leukemia (T-MPAL) is an exceedingly rare hematological neoplasm that accounts for less than 1% of acute leukemias. The prognosis for (T-MPAL) is poor and often associated with alkylating agents as well as topoisomerase-II inhibitors. We describe a case of therapy-related mixed phenotype acute leukemia following RCHOP therapy for primary cutaneous diffuse large B-cell lymphoma (PCDLBCL).

A 63 year-old female presented with several, large cutaneous lesions consistent with PCDLBCL. Staging revealed no bone marrow involvement or extracutaneous metastasis at the time of diagnosis. Given the patient’s extent of disease and the poor prognosis of PCDLBCL, she was treated with systemic chemotherapy which consisted of 6 cycles of RCHOP. She remained in remission for four years, until she presented with increased shortness of breath, night sweats, weakness and diffuse lymphadenopathy. Her presentation was initially concerning for recurrence of her PCDLBCL. Flow cytometry of the peripheral blood revealed 10% CD34 blasts with coexpression of CD117, bright CD7, cytoplasmic CD3, myeloperoxidase. The bone marrow biopsy was hypercellular at 80-90% and showed sheets of blasts. There was no evidence of DLBCL. Immunostaining of the bone marrow showed the tumor cells were positive for CD34, CD117, cytoplasmic CD3, CD7, myeloperoxidase, and TdT. Immunostains were negative for CD5, CD20, CD21, and CD79a. An inguinal lymph node biopsy also showed involvement by her new hematological malignancy. Karyotype was notable for 46, XX, t (8, 12) (q22, p13) with normal AML and MDS FISH panels. NPM1 and FLT3 were negative. Taken the patient’s clinical history together, she represented a case of T-MPAL, NOS likely secondary to cyclophosphamide and/or doxorubicin. To our knowledge this is the first case report involving T-MPAL with chromosome 12p13 abnormalities.
CAN PROSTATE TUMOR-DERIVED MICROVESICLES INDUCE NEOPLASTIC TRANSFORMATION OF STEM CELLS?

Saleem S*, Abd Elmageed ZY*, Thomas R*, Sartor O*, and Abdel-Mageed AB*

*Department of Urology, Tulane University School of Medicine, New Orleans, LA

Background: Obesity and increased body mass index have been directly correlated with prostate cancer (PCa) aggressiveness. Furthermore, mesenchymal stem cells (MSCs) have been implicated in enhancing tumor growth, though the underlying mechanisms involved remain elusive. On the other hand, there is emerging evidence that tumor-associated microvesicles (MVs), also known as exosomes, play a pivotal role in local and systemic cell–cell communication and contribute to the recruitment and reprogramming of constituents associated with tumor microenvironment.

Methods: In order to provide understanding of the mechanisms by which mesenchymal stem cells promote tumor growth and metastasis, and, whether cancer derived MVs are involved in this context, we tested the ability of PC cell-derived MVs to induce neoplastic transformation of patient-derived adipose tissue derived stem cells (pASCs). Tumor-tropic pASCs were enriched and treated with condition media (CM) or MVs of PC (C4-2B, PC-3) or normal prostate epithelial (RWPE1) cells. CM or MV-primed pASCs were examined for their ability to form prostate tumors in vivo. Tumor formation was validated by histopathologic analysis, mesenchymal-to-epithelial transition (MET), and expression of neoplastic and vasculogenic markers, karyotyping and PC specific markers. In our proposed experiments, we aim to test the ability of circulating MVs isolated from the blood of castration-resistant prostate cancer (CRPC) patients to induce neoplastic transformation of pASCs.

Results: Our preliminary data shows that PC cell microenvironment induces genotypic and phenotypic changes in pASCs and subverts them to undergo neoplastic transformation. Unlike normal counterparts, the pASCs primed with PC cell derived CM or MVs form prostate-like neoplastic lesions in primary and secondary recipients in vivo.

Conclusions: Our findings establish previously uncharacterized mechanisms for MV-pASC axis in tumor formation, phenotypic variations in mesenchymal and epithelial states in tumor microenvironment, and in promoting tumor burden and metastasis in cancer patients.
VON WILLEBRAND FACTOR AND FACTOR VIII ELEVATIONS – ASSOCIATIONS OF COMBINED ELEVATION OF BIOMARKERS WITH ADVERSE EVENTS AND POOR OUTCOMES AFTER ISCHEMIC STROKE


*Stroke Program, Dept. of Neurology, Tulane University School of Medicine, New Orleans, 70112
**Section of Hematology/Oncology, Dept. of Medicine, Tulane University School of Medicine, New Orleans, 70112
***Dept. of Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, 70112

Background: Despite clear roles of factor VIII (FVIII) and von Willebrand factor (vWF) in thrombosis, few studies have examined the relationship of these factors with ischemic stroke. We sought to determine if concurrent elevation in FVIII and vWF, rather than isolated elevation of FVIII, was associated with adverse events and outcomes.

Methods/Results: From our prospective stroke registry, patients admitted with acute ischemic stroke (AIS) 07/2008-10/2013 were included if both FVIII and vWF were measured during admission. We compared outcomes in patients with (++) and without (-) elevation in FVIII and vWF; regressions, adjusting for key covariates, analyzed outcomes with respect to FVIII(-)/vWF(-). The primary outcome was the modified Rankin Scale (mRS) score on discharge. Among the 1,453 cases in our stroke registry, 148 AIS patients met our inclusion criteria; 62 patients (41.9%) had FVIII(-)/vWF(-), 16 patients (10.8%) had FVIII(++)/vWF(-), and 51 patients (34.5%) had FVIII(++)/vWF(++) . In the fully adjusted model, patients with FVIII(++)/vWF(++) had increased odds of inpatient complications (OR 8.6, 95% CI 1.58-46.85, p=0.013) and neurological worsening (OR 3.2, 95% CI 1.18-8.73, p=0.022) than patients with FVIII(-)/vWF(-). Adjusted for age, baseline stroke severity and glucose, patients with FVIII(++)/vWF(++) had increased odds of poor functional outcome (mRS>2) (OR=2.87, 95% CI 1.16-7.06, p=0.021) as compared to patients with FVIII(-)/vWF(-).

Conclusions: Concurrent elevation in FVIII and vWF predicts higher odds of inpatient complications, neuroworsening, and worse functional outcomes for patients with AIS as compared to patients with normal levels of both clotting factors. Our findings suggest that FVIII and vWF levels may serve as clinically useful stroke biomarkers by providing risk profiles for patients with AIS.
Because the number of organs available for lung transplantation does not meet the growing demand, an alternative approach to traditional lung transplantation is needed. A recently developed technique known as detergent-mediated whole-organ decellularization is able to generate natural, three-dimensional extracellular matrix (ECM) scaffolds that are apt for lung tissue engineering. We have shown previously that lungs extracted from Sprague-Dawley rats with monocrotaline-induced pulmonary hypertension (MCT-PHT) can be efficiently decellularized as indicated by the removal of cells, reduction of DNA, and retention of ECM proteins. Here, to further investigate the effects of decellularization, we characterized the structural and mechanical features of MCT-PHT rat lung scaffolds as well as their potential for vascular recellularization. Polymer casting of the vasculature and airways of control and MCT-PHT rat lungs indicated that structural features such as capillaries and alveolar sacs were well-retained in control and MCT-PHT lungs after decellularization; however, microCT visualization of MCT-PHT rat lungs indicated that the vasculature was narrowed as a result of PHT pathogenesis, and this characteristic was unchanged by decellularization. Mean arterial vessel diameters of representative decellularized control and MCT-PHT scaffolds were estimated to be 0.247 ± 0.160 mm and 0.152 ± 0.134 mm, respectively. Quasi-static pressure-volume loops were used to evaluate the behavior of decellularized lungs during mechanical ventilation. A syringe pump was used to ventilate the lungs with a tidal volume of 10 mL/kg of rat body weight and a frequency of 1 breath/min. Compliance values were calculated from the slope of the inhalation curves during initial filling of the lungs. Decellularized control lungs and decellularized MCT-PHT lungs had similar compliance values and, hence, had similar opening pressures (0.0696 ± 0.0044 mL/mm Hg and 0.0696 ± 0.0024 mL/mm Hg, respectively; p=0.99503).

Control rat lung scaffolds were seeded with human umbilical vein endothelial cells (HUVECs) to develop an optimal vascular recellularization strategy. Ten million HUVECs were seeded into the vasculature of control rat lung scaffolds by gravity. The seeded scaffolds were then cultured in a sterile, sealed bioreactor for 3 days with continuous perfusion of endothelial growth media at 1 mL/min. Hematoxylin and eosin (H&E) staining of vascular-seeded, bioreactor-cultured lung tissue indicated that turbulent perfusion conditions may cause damage to seeded cells. These data show that decellularized MCT-PHT rat lung scaffolds have similar airway mechanics as control scaffolds, but have narrowed vasculature. Further research will focus on complete vascular recellularization of MCT-PHT rat lung scaffolds.
A CANINE MODEL OF DYSTONIA TO STUDY MEGDEL SYNDROME

Scott KJ, van Asbeck E, Kozicz T, Morava E

Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA

MEGDEL syndrome is an autosomal recessive disorder caused by mutations in SERAC1 and characterized by 3-methylglutaconic aciduria (3-MGAuria), deafness, encephalopathy and Leigh-like syndrome. SERAC1 acts as a protein mediator of phospholipid exchange at the mitochondrial-associated ER membrane with critical roles in maintaining normal mitochondrial function. Next-generation sequencing identified SERAC1 missense mutations in a spontaneous canine model with extremely similar phenotypes to MEGDEL syndrome. This animal model may serve as a useful tool for further studies of this disease. Herein we report on the clinical characteristics of four human patients confirmed to have MEGDEL syndrome and compare their presentations to the phenotype of a canine homozygous for a SERAC1 missense mutation. Clinical investigations included neuroimaging assessments, a comprehensive metabolic panel, mitochondrial function studies and mutation analysis. Patient and canine fibroblasts were stained for SERAC1 to evaluate expression and cellular localization as well as mitochondrial 39S ribosomal protein L2 (MRPL2) to evaluate mitochondria in fibroblasts. All patients were confirmed to have muscle weakness, spasticity, progressive dystonia, Leigh-like syndrome and cerebellar involvement. Metabolic alterations included 3-MGAuria, lactic acidemia and low normal cholesterol. Mitochondrial complex I and IV deficiencies were confirmed in the patients. Mutation analysis revealed all four patients to be homozygous for missense mutations. The animal model phenotype overlapped greatly with the patient phenotype, including progressive dystonia and Leigh-like syndrome, but it did not have 3-MGAuria. SERAC1 and MRPL12 stains in patient fibroblasts were similar to affected canine fibroblasts, which exhibited no significant difference to a control cell line. Despite the absence of 3-MGAuria in the canine model, we have noted remarkable similarity between the human and canine phenotypes of SERAC1 missense mutations. Our findings suggest that the animal model can be used for studying MEGDEL syndrome, especially basal ganglia involvement, dystonia and other neurodegenerative symptoms. Further studies include the evaluation of 3MGA excretion under different conditions and studying mitochondrial function in tissue samples and fibroblasts in the animal model of SERAC1 mutations.

This work was supported by The Hayward Foundation.
Parallel changes in the urinary excretion of ANG II and angiotensinogen (AGT) and kidney ANG II levels in slowly progressive ANG II-dependent hypertension

David Sigmon, Dale M. Seth, Akemi Sato, Porcha D. Davis, L. Gabriel Navar, and Kenneth D. Mitchell. Department of Physiology, Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA 70112

Previous studies have demonstrated that the urinary excretion of ANG II and AGT, and kidney ANG II levels are markedly elevated in ANG II-dependent malignant hypertension associated with marked renal injury. The present study was performed to determine the temporal changes in urinary ANG II and AGT excretion and kidney ANG II levels in a model of slowly progressive ANG II-dependent hypertension in Cyp1a1-Ren2 transgenic rats with inducible expression of the Ren2 renin gene. Male Cyp1a1-Ren2 rats (n=6/group) were induced to develop slowly progressive ANG II-dependent hypertension by dietary administration of indole-3-carbinol (I3C, 0.15% wt/wt) for 14 days. Conscious systolic blood pressures (SBP) increased from 140±8 to 176±9 mmHg (P<0.05) by day 4 of I3C induction, whereas the urinary excretion of ANG II and AGT both increased by day 3 of I3C induction (32.1±3 to 64.8±6 fmol/hr and from 123±25 to 437±75 ng/day, P<0.05 in both cases). Both urinary ANG II and AGT excretion continued to increase (P<0.01) and averaged 120±5 fmol/hr and 1384±514 ng/day, respectively, by day 13 of induction. Similarly, kidney ANG II levels were increased by day 13 of induction (1042±219 vs. 349±96 fmol/g, P<0.05). SBP, urinary ANG II and AGT excretion and kidney ANG II levels promptly decreased to control levels following termination of dietary I3C administration (to 127±2 mmHg, 60±5 fmol/hr, 83±34 ng/day, and 473±185 fmol/g, respectively, P<0.01 in all cases). The present findings demonstrate that the urinary excretion of ANG II and AGT increases progressively, and that total kidney ANG II levels are increased, following induction of ANG II-dependent hypertension and decrease within 3 days to control levels following termination of dietary I3C administration. The data also indicate that the intratubular generation of ANG II increases progressively following induction of ANG II-dependent hypertension and decreases promptly to control levels following termination of induction of ANG II-dependent hypertension.
According to the National Cancer Institute and the National Institute of Diabetes and Digestive and Kidney Diseases, the incidences of renal cell carcinoma (RCC), chronic kidney disease, and end-stage renal failure are significantly and steadily increasing (USRDS 2013 Annual Data Report; Howlader N, 2011). Approximately 10% of the U.S. adult population suffers from renal diseases. Collectively, these diseases are etiologically complex with environmentally mediated risk factors contributing to an estimated 90% of cases. Likewise, the influence of xenobiotic exposure on the initiation and progression of renal diseases remains largely unknown. To address this gap in our understanding, the Tox21 initiative has set forth goals to develop improved in vitro models with which to investigate human conditions, such as renal disease, that may be promoted by toxicant exposure (Tice et al., 2013).

In order to elucidate the mechanisms by which the heavy metal and nephrotoxicant cadmium (Cd) acts as a co-carcinogen, we aimed to characterize a newly developed cell line derived from the renal proximal tubule epithelial cells (RPTEC) of a healthy human male donor. The RPTEC/TERT1 cell line has been immortalized using the human telomerase reverse transcriptase (hTERT) catalytic subunit and does not exhibit chromosomal abnormalities (Evercyte Laboratories). Controlled exposure experiments were designed to demonstrate toxicological responses, DNA repair capacity, and mutagenicity in these cells, which will serve as a model for renal cell carcinogenesis. We have conducted single compound as well as binary mixture experiments with the common environmental carcinogens, Cd and benzo[a]pyrene (B[a]P). Our studies are the first to provide information regarding toxicological responses in this novel cell line. RPTEC/TERT1 cells exhibit compound specific gene expression responses when exposed concentrations as low as 1nM B[a]P and 1µM Cd. A significant increase in the expression of genes coding for B[a]P metabolizing enzymes (CYP1A1, CYP1B1) occurred in a dose- and time-dependent manner. We verified the activity of these enzymes using the EROD activity assay. Likewise, a significant increase in the heavy metal responsive gene, MT2A, was observed following exposure to Cd. The presence of BPDE-DNA adducts confirms that the RPTEC/TERT1 cell line responds to B[a]P in a manner consistent with what is known regarding these cells in a normal, healthy kidney. Future studies will be conducted to test mutagenesis under conditions of co-exposure to Cd and B[a]P. We hypothesize that Cd inhibits DNA repair processes, therefore, causing BPDE-DNA mutations to become fixed in the genome which may lead to carcinogenesis. These studies will help scientists better understand the initiating events that may promote carcinogenesis in normal, healthy human cells.

This work was supported by the Baton Rouge Area Foundation and the National Institutes of Health [grant number 5U19ES020677].

Solivan AE,* Harville EW, Buekens P
(Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70122)

Objective: To examine causes of death of infants in the postneonatal period (days 28-365) in adolescent mothers compared to adult mothers. Methods: All successfully matched postneonatal deaths from the linked birth-infant death data from the National Center for Health Statistics from 1999-2006 were analyzed with standardized weights applied. Underlying cause of death was grouped into 11 broad categories. Women were divided into adolescent (less than 20 years old) or adult (more than 19 years old). Logistic regression was used to compute adjusted odds ratios (aORs), adjusted a priori for race and maternal education. Results: Postneonatal deaths accounted for 33% of infant deaths (n=74,289). Of those deaths, 19% were to adolescent mothers (n=13,960) and 81% were to adult mothers (n=60,329). The leading cause of death to postneonates was SIDS (26.5% adolescent; 22.0% adult). Postneonates of adolescent mothers were more likely to die from SIDS (aOR 1.28, 95% CI 1.21-1.36), accidents (aOR 1.28, 95% CI 1.21-1.36), and assault (aOR 1.59, 95% CI 1.44-1.74) than adult mothers. Postneonates of adolescent mothers were less likely to die of a non-infectious disease (aOR 0.76, 95% CI 0.72-0.80), a complication of the perinatal period (aOR 0.79, 95% CI 0.73-0.85), or a birth defect (aOR 0.63, 95% CI 0.60-0.67) than postneonates of adult mothers. There were no significant differences based on maternal age for cause of death due to infections, including respiratory infections; and other external causes not previously classified. Conclusions: Research should focus on identifying the underlying causes of preventable deaths in adolescent mothers.
Targeting CD133(+) hepatic carcinoma cells by inhibiting glycolytic metabolism

Kyoungsub Song*, Hyunjoo Kwon*, Chang Han*, Tong Wu*

Department of Pathology and Laboratory Medicine, School of Medicine, Tulane University, New Orleans, LA

Cancer stem cells (CSCs) are responsible for tumor growth and resistant to chemotherapeutic cancer treatment. A metabolic pathway including glucose metabolism is an important diagnostic and therapeutic target for cancer treatment. However, glucose metabolism is poorly understood in liver cancer stem cells. Therefore, we aimed to explore the differences of glucose metabolism between CSCs and non-CSCs and their underlying mechanisms. In this study, we isolated CD133(+) populations from human hepatoma cell line PLC/PRF/5 and characterized CSCs properties by spheroid formation and several stem cell markers including CD44, EpCAM, OCT4 and KLF4. Interestingly, CD133(+) population showed a high expression level of glycolytic enzymes such as Glut1, HK1, PGAM1 and PDK4. In contrast, expression of gluconeogenetic enzymes (G6Pase, Pepck) were significantly lower in the CD133(+) population. Extracellular acidification rate (ECAR), which is an indication of lactic acid production from glycolysis, was significantly higher in CD133(+) cells compared to CD133(-) cells. Mechanistically, we found that the levels of miR-122 were significantly decreased in CD133(+) cells compared to CD133(-) cells. Ectopic expression of miR-122 decreased ECAR and spheroid formation in CD133(+) cells. We also demonstrated that PDK4 is a direct target of miR-122 as transfection of miR-122 mimic markedly reduced both mRNA and protein levels of PDK4. Treatment of dichloroacetate (DCA), which is PDK inhibitor, significantly inhibited spheroid formation in CD133(+) cells. Furthermore, CD133(+) cells were more sensitive to DCA treatment than CD133(-) cells, whereas CD133(+) cells were resistant to sorafenib treatment. Taken together, these results suggest that enhanced glycolysis is associated with CD133(+) CSCs character and inhibition of glycolysis through miR-122 or PDK4 could be a potential therapeutic target for liver CSCs.
Dot1/H3K79 PATHWAY MEDIATES DEFECTIVE URETERIC BUD(UB) BRANCHING LEADING TO RENAL HYPODYSPLASIA(RHD) IN PRORENIN RECEPTOR (PRR) PRRUB/- MICE

Song Renfang, Riedl Lindsay, Yosypiv Ihor V.

Department of Pediatrics, Hypertension and Renal Center of Excellence Tulane University Health Sciences Center, New Orleans, LA

Purpose of Study: Dot1 is histone methyltransferase specific for Histone 3 lysine 79 (H3K79) that is important for differentiation of collecting duct (CD) cells. Targeted deletion of the Dot1 in the CD principal cells (PCs) in mice represses the acquisition of PC phenotype resulting in polyuria (Wu. JASN, 2013). We tested the hypothesis that RHD and polyuria observed in mice that lack the PRR in the UB lineage (PRRUB/-) (Song. PLoS ONE, 2013) is due to reduced Dot1/H3 dimethyl K79 (H3m2K79) expression.

Methods Used: Mutant [Hoxb7Cre+PRRflox/flox (PRRUB/-), n=3] and control (PRRUB+/+, n=3) mice were studied on embryonic (E) day E17.5. Dot1 mRNA and protein expression in the kidney was studied by real-time qRT-PCR and immunohistochemistry, respectively. H3m2K79 protein expression was determined by immunohistochemistry and Western blot analysis. The intensity of H3m2K79 and Dot1 immunoreactivity, normalized for surface area of the kidney section, was examined by Slidebook 4.1 software.

Summary of Results: Kidney section surface area was smaller in the mutant compared to control mice (220600±20120 vs. 533800±72170 pixels, p<0.05). Dot1 mRNA levels were decreased in mutant compared to control mice (0.68±0.06 vs. 1.0±0.01, p<0.01). Dot1 and H3m2K79 immunostaining was reduced in the mutant vs. control kidneys (Dot1: 0.62±0.03 vs. 1.0±0.01, p<0.05; H3m2K79: 0.64±0.04 vs. 1.1±0.01, p<0.05). Western blot analysis revealed decreased H3m2K79 protein levels in mutant compared to control kidneys (1.0±0.06 vs. 1.5±0.02, p<0.05).

Conclusions: We conclude that reduced H3m2K79 methylation by Dot1 in the UB of PRRUB/- mice contributes, in part, to RHD and polyuria observed in these mice.
HIGH-THROUGHPUT DISCOVERY OF PEPTIDE ANTIBIOTICS: A DELICATE BALANCE BETWEEN ANTIMICROBIAL POTENCY, SOLUBILITY AND TARGET SELECTIVITY

Starr CG*, He J*, and Wimley WC*

*Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA 70112, United States

Antimicrobial peptides (AMPs) are key components of the innate immune systems of many organisms. AMPs function by permeabilizing microbial membranes, giving them an important advantage over conventional antibiotics as they may elude the selection of drug-resistance. Therefore, there has been increasing interest in engineering new AMPs and improving their bioactivity over the last three decades. Yet the lack of obvious structure-function relationships or molecular design principles has obstructed the development of new AMPs. To circumvent this roadblock, we are developing a high-throughput approach to select AMPs that are optimized in all of the critical factors simultaneously. Previously in our lab, we identified a group of broad-spectrum antimicrobial peptides from a synthetic peptide library. Here, we used one of the broad-spectrum AMPs *ARVA, (RRGWALRLVLAY) to study the potency, selectivity and mechanism of action of AMPs in the presence of concentrated human erythrocytes (10^9 cells/ml) which mimics the in vivo milieu. *ARVA, and other AMPs, lose antimicrobial activity in concentrated erythrocytes. We developed a method to make direct measurements of peptide binding to cells which showed that loss of activity is due to weak host cell binding, coupled with the large mass excess of host cell vs. bacterial cells under physiological conditions. To identify AMPs with clinically-relevant activity, we are developing a novel, orthogonal high-throughput screen in which we select simultaneously for 1) peptide solubility; 2) lack of host cell lysis; 3) sterilization of a Gram positive microbe in the presence of concentrated erythrocytes, and 4) sterilization of a Gram negative microbe in the presence of concentrated erythrocytes. Our results show that rational library design and high-throughput screening is a promising approach to identify AMPs that have the needed balance between antimicrobial potency, solubility and target cell selectivity.
ADIPOSE-DERIVED STROMAL/STEM CELL THERAPY ACCELERATES HEALING OF ISCHEMIA-REPERFUSION INDUCED PRESSURE ULCERS IN YOUNG AND AGED ANIMALS


*Center for Stem Cell Research and Regenerative Medicine, Tulane University School of Medicine, New Orleans, Louisiana; **Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Florida, Gainsville, Florida; ***Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland; ****LaCell LLC, New Orleans, Louisiana

Pressure ulcers (PU) are localized injuries to areas of skin or underlying tissue as a result of ischemia reperfusion. The prevalence of pressure ulcers increases with age due to an increase in the fragility of blood vessels and connective tissue and the loss of adipose tissue and muscle, reducing the threshold for pressure-induced injury. With the aging demographic of the world’s population, a greater number of individuals will be at risk for the development of PU injuries in the next decade. Debridement, antibiotics, and hyperbaric chambers are the first line treatment for late stage PU, yet the morbidity and mortality associated with late stage PU remains high due to the difficulty in addressing the damage to the underlying muscle and fascia. Therefore, new therapies aimed at regenerating the underlying tissue in PU are necessary. Adipose-derived stromal/stem cells (ASCs) have previously been shown to accelerate the healing of full thickness skin wounds in diabetic mice through the release of angiogenic and reparative factors into the local environment. Herein, ASCs were harvest from inguinal white adipose tissue of C57Bl/6 mice transgenic for the green fluorescent protein. To assess efficacy of ASCs, PBS or 1 x 10^6 ASCs were injected subcutaneously into pressure ulcer wounds in young (2 month) and aged (20 months) animals following PU induction. ASC therapy significantly accelerated wound closure rates, improved tissue histology, and altered the expression of key reparative genes, such as TGF-beta, PDGF-beta, VEGF, HGF, MMP-9, and MMP-13. Histological analyses demonstrate reduced infiltration of immune cells, increased collagen deposition, and reduced hypertrophy of the epidermis after treatment with ASCs. Together, these results suggest that ASCs can accelerate the healing of full thickness wounds in young and aged mice and provide support for the translation of this novel cell based therapy into clinical practice.

The work is supported by the National Institute of Aging of the National Institute of Health under award number 1-R43-AG042904-01.
A NOVEL ANTISENSE RNA TRANSCRIPT WITHIN THE EBV BAMHI A REGION ACTS AS A MICRORNA SPONGE; IMPLICATIONS IN VIRAL REACTIVATION


*Department of Pathology, Tulane University, New Orleans, LA, **Tulane Cancer Center, New Orleans, LA

Epstein-Barr virus (EBV) undergoes reactivation, a process associated with the orchestrated cascade of viral gene signing involved in the production of infectious virions. This lytic cascade is known to require the expression of approximately 80 previously annotated lytic genes. However using strand specific RNA-seq, we have previously studied EBV reactivation and have identified the expression of an additional several hundred novel viral transcripts, most of which are transcribed antisense to previously annotated genes. Among these new transcripts was one that was found in the EBV BamHI A region which is antisense to the cluster 1 group of EBV encoded BamHI A microRNAs. Using strand specific RNA-seq along with strand specific PCR validation, we were able to confirm the expression of this transcript upon reactivation of EBV. Additionally, fluorescence in-situ hybridization and PCR studies identified these transcripts localizing within the cytoplasm of EBV infected human B-cells. Finally, TaqMan Assay studies of the EBV BART microRNAs associated with the BamHI A antisense transcript identified an enrichment of the EBV cluster 1 BART microRNAs and not cluster 2. These studies raise the possibility that these antisense transcripts are involved in reactivation through the inhibition of the BART cluster 1 microRNA function.

This work was supported by National Institutes of Health grants R01CA124311 and R01CA138268 to EKF and a Ruth L. Kirschstein National Research Service Award F30CA177267 from the National Cancer Institute to MJS.
After the nuclear accident of April 26, 1986 in Chernobyl, children in the Narodichesky region participated in a yearly medical screening to evaluate their health status. The accident exposed the children to radiation; Cesium-137 was one of the radionuclides present. The nuclear accident exposed people in the Narodichesky region to acute amounts of Cs137, mainly from consuming cow’s milk and eating local grown foods (Cs137 was found in the soil). The procedures used to investigate the association of Cs137 intake and metabolic system was a “natural experiment” approach and longitudinal prospective cohort. These methods were performed on 923 children using 5,554 repeated measurements from 1993-1998. An Index was created by combining variables from the liver, gallbladder, and pancreas. Cs137 exposure was categorized into 5 quintiles. By examining the association between Cs137 and parts of the metabolic system it will be determined if children exposed to radiation have increased health effects. The data being used was from a longitudinal prospective cohort from 1993-1998. By using the odds ratio it will be determined if there is an association between Cs137 and the increased amount of abnormalities present. The findings suggest that Cs137 significantly affected the liver and gallbladder but it is not observed in the pancreas (OR=1.04 95%CI 1.01-1.06). The results suggested that public health concern for the amount of children considered unhealthy or having health effects due to the Cs137 exposure. These findings are unique and suggest significant liver and gallbladder disease consequences for children chronically exposed to low-dose radioactive contaminants such as those found downwind of the Chernobyl Nuclear Power Plant.

This work was supported by a fellowship from Center for Gulf Coast Environmental Health Research, Leadership and Strategic Initiatives.
RECOMBINANT ANTIBODIES THAT RECOGNIZE ALKYLATED POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

Sun Y*, Ban B**, Ansari GAS***, Blake DA*

*Department of Biochemistry & Molecular Biology, School of Medicine, Tulane University, New Orleans, LA
**College of Pharmacy, Xavier University of Louisiana, New Orleans, LA,
*** Department of Biochemistry & Molecular Biology, University of Texas Medical Branch, Galveston, TX.

Polycyclic aromatic hydrocarbons (PAHs) remaining in the weathered oil are both the most toxic/carcinogenic pollutants and the most difficult to bioremediate. The accurate detection and measurement of PAHs in the natural environment are therefore critical to environment protection and human health. Our laboratories employed phage/yeast display technologies to develop antibodies that specifically recognize alkylated PAHs, which are derived almost exclusively from oil spills and seeps. 2-Methylphenanthrene (2-MP) and 2,7-dimethylphenanthrene (2,7-DMP) were conjugated to protein and used as immunogens. Libraries of single chain antibodies (scFvs) were constructed from the cDNA of mouse immune cells, and scFv libraries were displayed on the surface of phage particles for selection of antibodies that specifically recognized methylated phenanthrenes.

After seven rounds of selection, monoclonal scFv’s were selected based on their unique sequence and their binding properties. Individual clones (104) were analyzed by clone PCR. Thirty-seven (35.6% out of total) of the selected clones had full-length sequences (800bp). Full-length clones were further analyzed by functional analysis (phage ELISA) and BstNI fingerprinting. Twenty-seven clones bound to 2mp-BSA. Of these clones, 1C1 had a unique fingerprint, and 26 showed similar BstNI fingerprints (1C4-like patterns). Other 10 clones were considered to be non-specific binders (ELISA signals less than three-fold above background). The 2 positive clones (1C1 and 1C4) were expressed as soluble scFv’s and purified. Analysis of 1C1 showed that it preferentially bound to phenanthrenes over pyrene. Analysis is now underway to determine the ability 1C1 and 1C4 to recognized soluble phenanthrene and methylated phenanthrenes. Further analysis and incorporation of these antibodies into our available immunosensor technology would provide a novel and convenient way to detect alkylated PAHs arising from crude oil.

Supported in part by the NIEHS (U19ES020677).
Cognitive aging is associated with a decline in some aspects of attention control such as conflict processing. The Simon effect indexes conflict between stimulus location, which is irrelevant, response locations to non-spatial information. Event-related potentials index neural responses associated with conflict detection (medial frontal negativity, MFN, ~250-500 ms) and later conflict processing (slow waves, 600-800 ms). The current study expands work on visual lexical conflict by examining age differences in auditory spatial conflict. Young (n=12, 18-30yrs) and healthy older (n=12, 60-80yrs) subjects performed an auditory Simon-task. Conflict is evident by longer reaction times when the sound and response are on opposite left-right sides (incompatible-trials) vs. the same side (compatible-trials). Older individuals had a larger Simon effect for reaction time measures (p<.05). The MFN for incompatible trials was more negative from 250-350 ms (p<.01) and did not differ between age groups. The onset of slow waves was delayed by ~150 ms in older subjects, and frontal slow waves were more negative on incompatible trials for young (p<.03) but not older (p<.60) subjects. However, right parietal slow waves only in older subjects differed between trial types (p<.01). Comparable MFN amplitudes among groups suggest that age differences in auditory spatial may conflict arise after conflict detection. Conflict was indexed by slow waves at frontal sites in the young and right parietal sites in older subjects. This shift from prefrontal to right parietal sites may reflect compromised prefrontal function in aging and/or greater processing of irrelevant spatial information in right parietal areas.
Objective. Experimental stroke studies have observed greater infarct injury in animals with deletions of endothelial nitric oxide synthase (eNOS) but reduced infarct size in neuronal nitric oxide synthase (nNOS) knockout mice. Interestingly, pharmacological inhibition of NOS reported varying impact on brain infarct size following stroke. Studies in our laboratory have identified the expression of nNOS in rat brain microvascular endothelial cells; however, the functional role of nNOS in human brain microvascular endothelial cell (hMECs) has never been examined. Our objective was to identify the nNOS in hMECs and study its role in the regulation of mitochondrial function and response to anoxic injury.

Methods and Results. Immunohistochemistry identified von Willebrand factor, eNOS, and nNOS in MECs in primary hMECs. Similar to primary rat brain MECs, the nNOS immunoreactivity to three antibodies raised against different sequences of nNOS was observed in the cytoplasm and also in the nucleus when cells were permeabilized. Oxygen consumption rate (OCR) measurements made from hMECs treated for 3 hours with selective inhibitors of nNOS (N-ω-Propyl-L-arginine; NPA) and eNOS (L-N5-(1-Iminoethyl)ornithine; NIO) revealed that mitochondrial reserve respiratory capacity was enhanced by nNOS inhibition but diminished by eNOS inhibition. OCR in, pmole/min/viability index was 721±12 with no treatment and was increased to 933±10 following 50 nmol/L NPA treatment (n=3 each; p<0.05). In contrast, OCR was reduced by 1 µmol/L NIO and 10 µmol/L NPA (a concentration that nonspecifically inhibits all NOS isoforms) to 112±43 and 167±74, respectively (n=3 each; p<0.05 versus untreated cells). Drug treatment of hMECs for 3 h followed 24 h later by cell viability measurements showed that nNOS inhibition with 1 µmol NPA increased cell proliferation (20±2.4% versus control, n=3 96-well plates; p<0.05). Conclusions. We identified immunoreactive nNOS in primary human brain MECs. Pharmacological inhibition of nNOS in MECs enhanced mitochondrial capacity and promoted cell proliferation. Thus, in MECs, nNOS appears to function distinct from eNOS and may even counteract eNOS actions.
THE EFFECT OF AGING ON MICROVASCULAR DENSITY AND ANGIOGENESIS

Sweat RS*, Chedister LO*, Sloas DC*, Stewart SA*, Murfee WL*

*Department of Biomedical Engineering, Tulane University, New Orleans, LA

Age related pathologies are commonly associated with vessel loss or excess vessel growth. An important question is whether or not angiogenesis, defined as the growth of new vessels from existing ones, is altered during aging. Angiogenesis in aged populations is commonly thought to be impaired. However, much of this supporting evidence is based on local changes in angi-regulator molecules. The objective of this study was to quantitatively compare microvascular network remodeling metrics during angiogenesis in adult versus aged rat strains. Mesenteric tissues from adult (9 months) and aged (24 months) male Fischer 344 rats were harvested according to 4 experimental groups: 1) Adult Unstimulated (n=4 rats, 16 tissues), 2) Aged Unstimulated (n=4 rats, 16 tissues), 3) Adult Stimulated (n=4 rats, 16 tissues), 4) Aged Stimulated (n=4 rats, 16 tissues). For stimulated groups, tissues were harvested 3 days post compound 48/80-induced mast cell degranulation stimulation. Unstimulated aged microvascular networks displayed larger mean vascular area per tissue area compared to the unstimulated adult networks. Following angiogenic stimulation, both adult and aged networks displayed similar increases in vascularized area and vessel length density compared to their respective unstimulated control groups, indicating that both adult and aged networks are capable of undergoing angiogenesis. However, capillary sprouting was significantly lower in stimulated aged networks compared with stimulated adult networks, supporting the hypothesis of impaired angiogenesis during aging. Interestingly, our results provide metric specific evidence for both comparable and impaired angiogenesis during aging and offer a possible explanation for conflicting results from the literature.

This work was supported by the Tulane Center for Aging and NIH-P20GM103629-02
PDGF RECEPTOR ANTAGONISM PREVENTS THE INCREASE IN KIDNEY ANGIOTENSIN II LEVELS IN ANGIOTENSIN II-DEPENDENT MALIGNANT HYPERTENSION

Thomson DA*, Seth DM*, Davis PD*, Mitchell KD*

*Department of Physiology, Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA.

Previous studies demonstrated that chronic administration of the PDGF receptor kinase inhibitor, imatinib mesylate, ameliorates the renal injury, improves renal hemodynamics, and prevents the augmented urinary angiotensin II (ANG II) excretion in transgenic rats [TGR(Cyp1a1Ren2)] with angiotensin (ANG) II-dependent malignant hypertension. The present study was performed to determine if chronic PDGF receptor blockade prevents the augmentation of intrarenal ANG II levels that occurs in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension. Male Cyp1a1-Ren2 rats (n=5/group) were induced to develop malignant hypertension by dietary administration of indole-3-carbinol (I3C, 0.3% wt/wt) for 10 days. One group was chronically treated with imatinib mesylate by oral gavage (60 mg/kg/d) starting 3 days before initiating I3C induction and maintained on imatinib for the duration of the study. Systolic blood pressures (SBP) were measured daily by tail-cuff plethysmography. On day 10 of I3C administration, rats were subjected to conscious decapitation and trunk blood was collected for measurement of plasma renin activity (PRA) and plasma ANG II levels. The kidneys were harvested for determination of total kidney ANG II levels. Dietary I3C resulted in pronounced increases in SBP (130±5 to 171±9 mmHg, P<0.05), PRA (64±6 vs. 5±1 ng ANG I/ml/hr, P<0.001), plasma ANG II levels (186±26 vs. 139±10 fmol/ml, P<0.05), and augmented total kidney ANG II content (550±31 vs. 253±24 fmol/g, P<0.001). Chronic imatinib administration did not prevent the development of hypertension (187±10 vs. 141±2 mmHg, P<0.001) or the increase in PRA (72±6 vs. 5±1 ng ANG I/ml/hr, P<0.001), but prevented the I3C-induced augmentation of intrarenal ANG II levels (285±128 vs. 253±24 fmol/g). The present findings demonstrate that PDGF receptor antagonism prevents the marked increase in intrarenal ANG II levels independent of changes in blood pressure that occur in ANG II-dependent malignant hypertension. The data also indicate that chronic elevations of intrarenal ANG II levels are not required for the maintenance of the elevated arterial blood pressure in ANG II-dependent malignant hypertension.
POLYFUNCTIONAL T LYMPHOCYTES ARE KEY PROTECTIVE RESPONSES IN SIVMAC239ΔGY CONTROLLER PIGTAIL MACAQUES


*TNPRC-Pathology, Tulane National Primate Research Center, Covington, Louisiana, USA; **SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA; ***University of Pennsylvania, Philadelphia, Pennsylvania, USA.

We have recently shown that disruption of the conserved motif GYxxØ in the SIVmac239 envelope cytoplasmic tail results in a virus that replicates to wild-type levels acutely while sparing mucosal CD4 T cells. Fourteen of sixteen pigtail macaques (PTM) inoculated with this virus, ΔGY, controlled infection with viral set points below 100 copies/ml (elite controllers) while two animals failed to control infection (progressors). To determine the role of T-cells in controlling ΔGY we examined SIV specific CD4 and CD8 T cell responses in peripheral blood mononuclear cells (PBMC) and intestinal lamina propria leukocytes (LPL) in the 16 PTM inoculated with ΔGY and 4 additional PTM inoculated with SIVmac239. T cell dynamics and SIV-specific CD4 and CD8 T cell responses in intestine and blood were evaluated at multiple time points after infection. The elite controllers were characterized by maintenance of memory CD4+ T cells and rapid development of SIV-specific responses including polyfunctional responses that persisted. Interestingly, these responses were greater in LPL than PBMC and in CD4 T cells than CD8 T cells. We also noted that the cytokine responses were dominated by IFNγ and TNFα in intestinal tissues. In contrast, the ΔGY progressors and the SIVmac239-infected PTMs showed declines in memory CD4 T-cells and few polyfunctional cytokine responses. The magnitude of the loss of CD4 T cells was much greater in the SIVmac239-infected PTM than in the ΔGY progressors and the cellular responses were also weaker. This study demonstrates remarkable control of viral replication in PTM that are highly susceptible to SIV induced disease that is associated with maintenance of central memory CD4 T-cells and strong polyfunctional CD4 and CD8 T-cell responses particularly in LPL.

Supported by PHS grants: OD011104, AI074362, AI0459008 and OD011124.
PILOT INVESTIGATION OF VIDEO-RATE STRUCTURED ILLUMINATED MICROSCOPY (VR-SIM) VERSUS TRADITIONAL HISTOLOGY FOR DIAGNOSIS OF PROSTATE CANCER

Tulman DB*, Wang M*, Kimbrell HZ**, and Brown JQ*
*Department of Biomedical Engineering, Tulane University, New Orleans, LA, USA
**Department of Pathology and Laboratory Medicine, Tulane University, New Orleans, LA, USA

The standard-of-care method to diagnose prostate cancer is imaging tissue following hematoxylin and eosin (H&E) staining. However, results from H&E staining cannot be reviewed until several days post-procedure, a process that can be time consuming for both technicians and pathologists. Furthermore, it is desirable to image large areas of tissue in a shorter, clinically relevant time frame when assessing tumor margins. We developed a VR-SIM system capable of rapidly imaging large tissue areas with high contrast and pathologically-relevant subcellular resolution. Here, we present preliminary data to validate the diagnostic capability of our VR-SIM system compared to review of H&E stained tissue. Known cancerous and non-cancerous prostate biopsy specimens were imaged using VR-SIM and later fixed and sectioned for H&E. A blinded pathologist reviewed the VR-SIM images and H&E slides to determine the presence of cancer and the level of confidence in the diagnosis (Figure 1). When each biopsy section was reviewed using both methods, the diagnosis of cancer/no cancer was matched in 23 of 30 (76.7%) of the biopsies. In a subset of images analyzed with optimized settings (n=16), ROC analysis yielded an area under the curve of .895. Results show promise for diagnostic co-registration between VR-SIM and H&E, though further optimization of the VR-SIM system settings and review of more samples is necessary. Ideally, VR-SIM can be optimized for the capability of imaging the entire surface of a prostate surgical section, yielding an image pathologists can quickly scan for tumor margins (Figure 2).

This work was supported by: NIH/NCI R21CA159936 and the Tulane University Bioinnovation Program, Tulane University School of Science and Engineering.

![Figure 1](image1.png)

Figure 1. Wide-field (No SIM), VR-SIM, and H&E section images of a prostate biopsy containing cancer. The widefield and VR-SIM images were collected on the intact biopsy, which was later fixed and sectioned for H&E. Skeletal muscle fibers and cancerous glands are observable against the normal stroma in the VR-SIM image.

![Figure 2](image2.png)

Figure 2. A) VR-SIM mosaic of a 4.7 x 3.9 x 1 cm cross-sectional slice of fresh human prostate stained with acridine orange. B) Zoom of tissue in the AEPS, consisting of a mix of smooth muscle and vessels. C) Zoom of area in B. D) Zoom of normal glandular tissue in the peripheral zone.
TGFβ SENSITIZES HEPATOCELLULAR CARCINOMA CELLS TO GROWTH FACTORS, PREVENTING SORAFENIB INDUCED APOPTOSIS

Ungerleider NA, Han C, Wu T

Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA

An upregulation in the transforming growth factor β (TGFβ) signalling pathway is associated with poor prognosis in hepatocellular carcinoma (HCC) patients. In this study, we aim to investigate the mechanisms by which TGFβ contributes to HCC tumor progression. Using the hepatoma cell line, PLC/PRF/5, we demonstrate that TGFβ increased expression of insulin-like growth factor 1 receptor (IGF1R), epidermal growth factor receptor (EGFR), and fibroblast growth factor receptor 1 (FGFR1). Furthermore, TGFβ pretreatment significantly increased cellular response to IGF1, IGF2, EGF, and FGF treatment, as measured by levels of pAKT and pERK. We hypothesized that this increase in growth factor sensitivity would render cells resistant to sorafenib, the only approved targeted HCC chemotherapeutic agent. To test this, we pretreated cells with TGFβ or left cells untreated for 48 hours then incubated the cells with sorafenib for up to 24 hours. We found that TGFβ pretreatment led to a reduction in apoptotic markers and increased cell viability in the presence of sorafenib, as measured by cell counting and WST-1 assays. Taken together, our data suggests that TGFβ can enhance the survival of HCC cells by upregulating cell sensitivity to pro-survival growth factors.

This work was supported by National Institutes of Health grants. Grant Numbers: CA106280, CA102325, CA134568, DK077776
REGULATION OF A NOVEL CALCIUM-SENSITIVE DICARBOXYLATE TRANSPORT PROCESS BY CALCIUM-SENSING RECEPTOR SIGNALING

Walker RW, Coleman-Barnett JA, Hamm LL, Hering-Smith KS

Urinary citrate is a potent inhibitor of nephrolithiasis as it prevents the formation of insoluble complexes between calcium and oxalate and other anions by complexation of calcium (Ca\(^{2+}\)). The concentration of citrate in the urine is determined by proximal tubule reabsorption by transport on the apical membrane through the sodium dicarboxylate cotransporter (NaDC1). We previously demonstrated a novel apical calcium-sensitive dicarboxylate transport process in an opossum kidney proximal tubule cell line (OK). Reducing extracellular Ca\(^{2+}\) from 1.2 mM to <60 µM results in a marked increase in citrate and succinate transport in OK cells. This process is likely not NaDC1 since we have previously determined that NaDC1 is not calcium-sensitive. The purpose of this study is to determine the role of the calcium-sensing receptor (CaSR) and its related signal transduction pathways in the regulation of calcium-sensitive dicarboxylate transport. Radioisotope uptake assays using \(^{14}\)C-citrate (Cit), or \(^{14}\)C-succinate (Suc) were performed in OK cells in normal (1.2 mM) and low (<60 µM) Ca\(^{2+}\) conditions. To test if CaSR is involved in the regulation of this transport process, 1 mM spermine (Sp), a type one agonist of CaSR, was added to the uptake buffer. Suc uptake was inhibited by Sp in both normal (0.067 ± 0.007 - 0.047 ± 0.005 pmol/well, p<0.0001), and low Ca\(^{2+}\) conditions (0.1 ± 0.01 - 0.07 ± 0.01 pmol/well, p=0.0004). To examine if CaSR regulates transport through PKC activation, OK cell monolayers were pre-incubated with 50 nM PMA for 30 min followed by Suc uptake. In normal Ca\(^{2+}\), PMA did not significantly change transport. In low Ca\(^{2+}\) conditions PKC activation inhibited Suc uptake (0.217 ± 0.013 - 0.179 ± 0.012 pmol/well, p=0.0004). To test if CaSR regulates Ca\(^{2+}\)-sensitive dicarboxylate transport through adenylate cyclase (AC) inhibition, OK monolayers were preincubated for 30 min with 50 µM of the AC inhibitor MDL-12,330A. Suc uptake was inhibited by MDL in both normal (0.088 ± 0.0075 - 0.048 ± 0.009 pmol/well, p=0.004), and low Ca\(^{2+}\) conditions (0.13 ± 0.009 - 0.078 ± 0.009 pmol/well, p=0.0011). To determine if inhibition of Ca\(^{2+}\)-sensitive dicarboxylate transport can be reversed by increasing cAMP, cells underwent 30 min pretreatment of 100 µM 8-Br-cAMP prior to Suc uptake. In normal Ca\(^{2+}\) conditions uptake was inhibited (0.118 ± 0.005 - 0.11 ± 0.0.006 pmol/well, p<0.016) and that there was no effect in low Ca\(^{2+}\) conditions. In conclusion, the activation of the CaSR using Sp caused inhibition in low Ca\(^{2+}\) conditions, however Sp also caused inhibition in normal Ca\(^{2+}\) conditions suggesting further enhancement of CaSR activity. PKC activation with PMA resulted in transport inhibition in low Ca\(^{2+}\) conditions only, suggesting that the CaSR may regulate the calcium-sensitive process specifically through the PKC pathway. Inhibition of AC by MDL-12,330A resulted in decreased dicarboxylate transport in both normal and low Ca\(^{2+}\) conditions. 8-Br-cAMP did not increase dicarboxylate transport in normal Ca\(^{2+}\) conditions suggesting that simply increasing cAMP in the cell may not be enough to reverse the effects of the CaSR. Thus the CaSR regulates calcium-sensitive dicarboxylate transport through the PKC pathway and it may also regulate Ca\(^{2+}\)-sensitive dicarboxylate transport through another pathway involving AC.
VIDEO-RATE STRUCTURED ILLUMINATION MICROSCOPY (VR-SIM) FOR RAPID POINT-OF-CARE PATHOLOGY

Wang M*, Schlichenmeyer TC*, Elfer KN*, Kimbrell HZ**, and Brown JQ*
*Department of Biomedical Engineering, Tulane University, New Orleans, LA, USA
**Department of Pathology and Laboratory Medicine, Tulane University, New Orleans, LA, USA

Even though permanent histopathology provides the gold-standard clinical reference for pathologic diagnosis, it is time consuming and cannot be completed within point-of-care timeframes for large specimens. In clinical settings such as for imaging of excised tumor specimens, the areas required to cover often exceed 100 cm², which poses a great challenge to fluorescent optical sectioning microscopy techniques. To address this challenge, we developed a Rapid Optical Sectioning Specimen Scanner (ROS³), based on incoherent SIM, specifically for rapid, high-area throughput fluorescence microscopy of intact surgical and biopsy specimens. We validate the ROS³ system performance metrics on bovine tissue and human prostate biopsy, and we demonstrate the feasibility of the system for rapid optical sectioning microscopy of large tissue areas in clinically-relevant timeframes. We achieved high resolution, optically-sectioned images of fluorescent samples at area-throughput rate up to 4.4 cm²/min at current scan stage speeds, with 1.3 mm lateral resolution. An image mosaic of 30.4-cm² bovine muscle sample stained with acridine orange is shown in Fig. 1 (AA), which is composed of 1,800 individual frames. Altogether, the total scan time for this sample, including stage translation, was 15 minutes, for an area-throughput rate of 2 cm²/min. Fig.1 (BB) shows a mosaic images of a human cancerous prostate biopsy stained with proflavine. Using this system, we achieved, to our knowledge, the fastest pixel sampling rates (126 MHz) demonstrated for optical sectioning using SIM to date. We were able to image a 30.4 cm² fluorescently-stained tissue area in 15 minutes at 1.3 mm resolution with a sub-optimum scan stage and a relatively slow SIM framerate (3.33 Hz).

This work was supported by NIH/NCI R21CA159936, Tulane University School of Science and Engineering.
Circadian and Melatonin Disruption by Exposure to Light at Night Drives Intrinsic Resistance to Tamoxifen Therapy in Breast Cancer


*Department of Structural and Cellular Biology, Tulane University School of Medicine, New Orleans, Louisiana  **Department of Surgery, Tulane University School of Medicine, New Orleans, Louisiana

Abstract

Resistance to endocrine therapy is a major impediment to successful treatment of breast cancer. 30% to 50% of patients with ER-α positive breast tumors fail to respond to endocrine therapy and, thus, display intrinsic/de novo resistance.

The molecular mechanisms of resistance to endocrine therapy agents are as diverse as they are intricate. Preclinical and clinical evidence links anti-estrogen resistance with tumor over-expression and/or activation of various families of receptor and non-receptor-associated tyrosine kinases. Recent evidence indicates that disruption of circadian time structure by night shift work and/or disturbed sleep-wake cycles may lead to a significantly increased risk of an array of diseases, including breast cancer. Rotating night shift workers have an increased risk for breast and prostate cancer, and Light Exposure at Night (LEN) was identified as the principal risk factor. We have previously reported that the circadian neurohormone melatonin, produced by the pineal gland at night, inhibits breast tumor and prostate tumor growth through the melatonin receptor (MT-1) by mechanisms including: (1) repression of phospho-activation of key signaling kinases (ERK and AKT), (2) inhibition of estrogen receptor alpha (ER α) transactivation, and (3) suppression of tumor cAMP levels and aerobic glycolysis (Warburg effect). In addition, melatonin’s inhibition of tumor linoleic acid (LA) uptake and its metabolism to the mitogenic signaling molecule 13-hydroxyoctadecadienoic acid (13-HODE) appears to play a critical role in the down-regulation of the epidermal growth factor and insulin-like growth factor-1 pathways. The present study was undertaken to determine if circadian/melatonin disruption by exposure of tumor-bearing animals to dim light at night (dLEN) would affect the tumor growth and drive intrinsic resistance to tamoxifen treatment in breast cancer. The study used our
circadian complete “tissue-isolated” breast tumor nude rat model. Female nude rats (Hsd:RH-Foxn1<sup>nu</sup>; n = 6/group) bearing tissue-isolated ER<sub>α</sub>/PR-positive MCF-7 human breast cancer xenografts were exposed to either a regular 12h/12h light/dark cycle, or a light/dark cycle that included dim LEN (dLEN) during the dark cycle (0.08 µW/cm<sup>2</sup> (0.2 lux) ). We demonstrate in tissue-isolated ER<sub>α</sub> + MCF-7 human breast cancer xenografts, grown in nude rats maintained on a light/dark cycle of LD 12:12 in which dim LEN (dLEN) is present during the dark phase (suppressed endogenous nocturnal melatonin), a significant shortening of tumor latency-to-onset, increased tumor metabolism and growth, and complete intrinsic resistance to 4OH-tamoxifen therapy. Conversely, in tumor xenografts from rats either housed in LD 12:12 (elevated endogenous nocturnal melatonin) or in dLEN and receiving nocturnal melatonin replacement, tumor latency-to-onset was significantly lengthened while tumor growth, nighttime tumor metabolism, and kinase and transcription factor phosphorylation were all suppressed. Thus, melatonin acts as both a tumor metabolic inhibitor and a circadian-regulated kinase inhibitor (CRKI) to re-establish the sensitivity of breast tumors to tamoxifen and drive tumor regression. These results indicate that dLEN-induced circadian disruption of nocturnal melatonin production lead to a complete loss of tumor sensitivity to tamoxifen.

#Equal contribution
This work is supported by Army Department of Defense grant (to S.M.H.), the National Institutes of Health Grant R21CA129875-04 (to D.E.B.),
IL-17 represses p53-dependent ribosomal stress in lung tumor cells

* Department of Pathology and Laboratory Medicine, **Department of Microbiology and Immunology, *** Section of Pulmonary Diseases, ****Heart and Vascular Institute, School of Medicine, Tulane University, New Orleans, LA

Purpose: Lung cancer is the leading cause of cancer deaths worldwide. One of the most frequent genetic alternations in lung cancer occurs in the p53 tumor suppressor gene. We previously reported that overexpression of IL-17 promoted rapid growth of mutant K-Ras-expressing lung tumors in mice. Our current work investigates the consequences of IL-17 overexpression upon progression of lung tumors that harbor tumor-promoting mutations in both K-Ras and p53.

Methods: To test the effects of p53 status upon lung tumor promotion by IL-17, we administered 1x10^8 pfu IL-17-expressing adenovirus (Adv-IL-17) by oropharyngeal aspiration or an identical amount of GFP-expressing adenovirus (Adv-GFP) to 2 lines of lung tumor-bearing mice that differ only in p53 status. One line, K-Ras^LA1, possesses germ line mutations only in K-Ras, develops multifocal lung tumors by 8 weeks and survives a little longer than one year. The second line, K-Ras^LA1-SPC-DNp53, possesses the same mutation in K-Ras, but also expresses a dominant negative mutant version of human p53 in the lung epithelium. This latter line also develops lung tumors rapidly and has a median survival of about 7 months. Our studies in mice were complemented by in vitro studies in lung tumor cell lines using low dose actinomycin D (Act D; 5 nM) to induce p53-dependent ribosomal stress.

Results and conclusions: Adv-IL-17 administration significantly increased lung tumor growth in K-Ras^LA1 mice as measured by more tumor cells per lesion and higher tumor proliferation index. However, Adv-IL-17 failed to promote lung tumor growth in K-Ras^LA1-SPC-DNp53 mice. In lung tumor cells established from K-Ras^LA1 mice (mK-Ras-LE cells), treatment with IL-17 (10 ng/ml) had no effect on the basal level of p53, but modestly repressed p53 upregulation by Act D. In accord with this observation, IL-17 repressed Act D-mediated induction of both p21 mRNA and p21 promoter-reporter activity in mK-Ras-LE cells. Since the serine/arginine-rich splicing factor (SRSF1) is known to activate p53 by forming a complex with MDM2-RPL5, we next investigated the role of SRSF1 in this repression. Indeed, knockdown of SRSF1 reversed the effects of IL-17 upon p21 mRNA induction in Act D-treated cells. IL-17 partially reversed p21 mRNA induction by Act D in p53 knockdown cells restored with wild-type human p53. Studies are in progress to determine whether silencing Act1, an adapter molecule integral to IL-17 signaling, will antagonize IL-17-mediated p53 repression. Together, our results demonstrate that IL-17 stimulates lung tumor growth, at least in part by antagonizing the function of wild-type p53.
ω-3 polyunsaturated fatty acid Regulate the Expression of 15-PGDH through miR 26 a/b in Cholangiocarcinoma

Department of pathology and laboratory medicine, Tulane University

Lu Yao, Chang Han*, Tong Wu*

ω-3 fatty acids are polyunsaturated fatty acids with a double bond (C=C) at the third carbon atom from the end of the carbon chain. Previous report shows ω-3 polyunsaturated Fatty acid (ω-3 PUFA) suppresses cholangiocarcinoma development partially by interfering PGE2 synthesis, but the mechanism is not fully illustrated. Here we report ω-3 PUFA (DHA) up-regulated PGE2 deactivator—15-PGDH expression by inhibiting miR26a/b, which resulted in impaired tumor growth. Cholangiocarcinoma cell lines CCLP-1 and TFK-1 were treated with DHA, or stable transfected with Fat-1 gene. Data shows DHA or Fat-1 expression could significantly enhance 15-PGDH expression while have little effect on promoter activity. miR26a/b, which binds to 15-PGDH 3’UTR, were significantly down-regulated in DHA treated or Fat-1 expression cholangiocarcinoma cells. Overexpression miR26a/b greatly abolished 15-PGDH enhancement effect of ω-3 fatty acids. miR26a/b located in intron region of gene family CTDSP, their expression is intensely correlated and regulated by transcription factor c-myc. We found significant association between ω-3 PUFA treatment and c-myc expression and evidenced that ω-3 PUFA regulates miR26a/b expression through transcription factor c-myc. Finally, Tumor growth in vitro and in vivo confirmed ω-3 PUFA/miR26 regulatory axis influence on cholangiocarcinoma through 15-PGDH. Fat-1 expression undermined tumor growth while overexpression miR26 or 15-PGDH knocking-down reverse it. The finding further rationalized ω-3 PUFA in tumor therapeutic intervention, and highlight miR26a/b and 15-PGDH as potential targets for prevention and treatment of human cholangiocarcinoma.
Arsenic Trioxide inhibits EBV reactivation and promotes cell death in EBV-positive lymphoma cells
Qinyan Yin, Mark Sides, Fayong Luo, Chunmin Dong, Yan Zhuang, Christopher H. Parsons, Erik K. Flemington, Cecile Sanchez, Joseph A. Lasky

Department of Medicine SL9, Section of Pulmonary Disease, Tulane University School of Medicine. 1430 Tulane Ave. New Orleans, LA 70112, USA

Abstract
Epstein Barr Virus (EBV) is associated with hematopoietic malignancies, such as Burkitt’s lymphoma, post-transplantation lymphoproliferative disorder, and diffuse large B-cell lymphoma. Since EBV is the critical element for causing these tumors, targeting the virus is a plausible therapeutic cancer strategy. Current approaches for EBV-associated lymphoma include chemotherapy to destroy the cancer cells, however, normal cells are also injured. Indeed most current therapies for EBV-associated lymphomas do not differ from the EBV-negative lymphomas. This research is focused on EBV-specific cancer therapy.

Our research shows that low dose arsenic trioxide (ATO) inhibits EBV gene expression as well as EBV genomic replication. EBV spontaneous reactivation starts as early as 6 hours after re-suspending EBV-positive Mutu cells in RPMI media in the absence of ATO, but not in cells cultured with ATO. ATO’s inhibition of EBV spontaneous reactivation is dose dependent. The expression of EBV immediate early gene Zta and early gene BMRF1 is blocked by ATO (0.5nM to 2nM) in EBV latency type I cells (Mutu and Rael) and EBV infected PBMC cells. The combination of ATO with ganciclovir further diminishes EBV gene expression. The ATO-mediated reduction of EBV gene expression can be rescued by co-treatment with the proteasome inhibitor, MG132, indicating that ATO promotes ubiquitin conjugation and proteasomal degradation of EBV genes. Co-immunoprecipitation assays with antibodies against ubiquitin or SUMO1 pull down more BMRF1 in ATO treated cell lysates. Concordantly, BMRF1 IP pulls down more ubiquitin protein in ATO treated cells. Furthermore, MG132 reverses the blocking effect of ATO on TPA-, anti-IgM- and TGF-beta-mediated EBV reactivation. Thus, mechanistically ATO’s inhibition of EBV gene expression occurs via the ubiquitin pathway. Moreover, ATO treatment results in increased cell death in EBV-positive Mutu/Akata cells compared to EBV-negative Mutu/Akata cells, as demonstrated by both MTT and trypan blue assays. ATO-induced cell death in EBV-positive cells is dose dependent, and time course experiments show that ATO exerts its maximum inhibition at day two. The combination of ATO with ganciclovir further enhances cell death in EBV-positive Mutu, Cl13, JY, Farage and PBMC. ATO-mediated inhibition of EBV gene expression results in cell death selectively in EBV-positive lymphocytes, suggesting that ATO may potentially serve as a drug to treat EBV-related lymphomas in the clinical setting.
INTERLEUKIN-18 IS A CRITICAL CYTOKINE TO PROMOTE LIFE-THREATENING ALLERGIC DISEASES

Zaidi AK, Dutt P, Shukla JS and Mishra A

Pulmonary Medicine, Eosinophilic Disorder Center, Tulane University School of Medicine, New Orleans, LA 70112.

Background: Interleukin (IL)-18 is an inflammatory cytokine that plays an important role in a number of inflammatory diseases including asthma, and food allergy. Granulocytes such as eosinophils, basophils, and mast cells are critical in asthma and other food allergen-induced inflammatory disorders.

Aim: Herein, we tested the hypothesis that IL-18 induction is sufficient to generate, growth and survival of eosinophils, basophils and mast cells from bone marrow progenitors in vitro as well as in vivo.

Methods: Bone marrow cells were cultured in the presence of IL-18, IL-5, IL-3, SCF, SCF + IL-5, SCF + IL-18, SCF + IL-3, IL-3 + IL-18 for 9 days. Cells were harvested and stained for eosinophil-, basophil- and mast cell-specific cell surface markers. Cells were then analyzed by flow cytometry. Furthermore, a pharmacological delivery and transgenic approach were also chosen to test the role of IL-18 in vivo for generating these granulocytes in promoting inflammatory disease pathogenesis.

Results: Interestingly, IL-18 generated eosinophils (CCR3+Siglec-F+), basophils (FceRI+CD49b+cdtk-) and mast cells (FceRI+CD49b-ckit-) similar to IL-3 or IL-5 from bone marrow cells cultured in the presence of IL-18 for nine days analyzed by cell surface expression for CCR3, Siglec-F, FceRI, CD49b, ckit. While the expression of cell surface markers on basophils and mast cells were similar to IL-3 generated basophils and mast cells, however, the expression of Siglec-F on IL-18 generated eosinophils are lower than IL-5 generated eosinophils. Notably, IL-18 either with IL-3 or IL-5 produced significantly higher percentages of these granulocytes compared to IL-18 alone. Additionally, we are now focusing our studies on the functional ability of IL-18 generated granulocytes (basophils, mast cells and eosinophils), compared to IL-3 or IL-5 generated granulocytes in vivo by using pharmacological or transgenic approach. Our initial data indicates that IL-18 generated granulocytes in vivo may be more pathogenic.

Conclusion: Taken together, our in vitro and in vivo data indicates that IL-18 induction is sufficient to promote severe life threatening allergic diseases and may be a target cytokine for future therapeutic interventions for food allergen-induced disorders.

This work was supported in part by the grants NIH R01 DK067255 (AM), and NIH R01 AI080581 (AM).
NEOPLASTIC REPROGRAMMING OF PATIENT-DERIVED ADIPOSE STEM CELLS BY PROSTATE CANCER CELL-ASSOCIATED EXOSOMES

Zakaria Y. Abd Elmageed\textsuperscript{1,4}, Yijun Yang\textsuperscript{1}, Raju Thomas\textsuperscript{1,4}, Manish Ranjan\textsuperscript{1}, Debasis Mondal\textsuperscript{2,4}, Krzysztof Moroz\textsuperscript{3,4}, Zhide Fang\textsuperscript{5}, Bashir M. Rezk\textsuperscript{1}, Krishnarao Moparty\textsuperscript{1,6}, Suresh C. Sikka\textsuperscript{1,2}, Oliver Sartor\textsuperscript{1,4}, Asim B. Abdel-Mageed\textsuperscript{1,2,4**}

Departments of Urology\textsuperscript{1}, Pharmacology\textsuperscript{2}, Pathology\textsuperscript{3} and Tulane Cancer Center\textsuperscript{4}, Tulane University Health Sciences Center; Biostatistics Program\textsuperscript{5}, School of Public Health, Louisiana State University Health Sciences Center; and Department of Urology\textsuperscript{6}, Southeast Louisiana Veterans Health Care System, New Orleans, Louisiana, USA, 70112

Background: Emerging evidence demonstrates that circulating mesenchymal stem cells (MSCs) are significantly higher in obese than lean cancer patients and are often recruited to tumor sites but their functional significance in tumor growth and disease progression remains elusive. Exosomes, small extracellular membrane-enclosed vesicles, are involved in cell-cell communications and modulation of cell biology, primarily through trafficking of genomic and proteomic materials into target recipient cells. The objective of this study is to investigate the role of prostate cancer (PCa) cell-derived exosomes in neoplastic transformation of PCa patients’ tumor-tropic MSCs.

Methods: Adipose tissue-derived stem cells (pASCs) were procured from obese PCa patients and their purity confirmed by FACS analysis. A transendothelial well system was used to enrich pASCs with high tumor homing potential. Exosomes were purified from castration resistant (C4-2B and PC-3) cells by differential ultracentrifugation and their purity was verified by cryo-transmission electron microscopy and PCR analysis. Induction of prostate tumors by pASCs primed with PCa-cell derived conditioned media (CM) or exosomes was examined in athymic nude mice. Tumor formation was verified by cytogenetic analysis and expression of epithelial, neoplastic, and vasculogenic markers by immunofluorescence analysis. Characterization of exosomes oncogenic “cargo” of PCa cells, including miRNAs, mRNAs and proteins was examined by miRNA array, qPCR and LC/MS-MS analyses, respectively.

Results: Herein we report that prostate cancer (PCa) cell microenvironment subverts PCa pASCs to undergo neoplastic transformation. Unlike normal ASCs, the pASCs primed with PCa CM formed prostate-like neoplastic lesions \textit{in vivo} and reproduced aggressive tumors in secondary recipients. The pASC tumors acquired cytogenetic aberrations and mesenchymal-to-epithelial transition (MET) and expressed epithelial, neoplastic, and vasculogenic markers reminiscent of molecular features of PCa xenografts. Our mechanistic studies revealed that PCa cell-derived exosomes are sufficient to recapitulate formation of prostate tumorigenic mimicry generated by CM-primed pASCs \textit{in vivo}. In addition to down-regulation of the large tumor suppressor homolog2 (Lats2) and the programmed cell death 4 (PDCD4), the tumorigenic reprogramming of pASCs was associated with trafficking by PCa cell-derived exosomes of oncogenic factors, including H-\textit{ras} and K-\textit{ras} transcripts, oncomiRNAs miR-125b, miR-130b, and miR-155 as well as the Ras superfamily of GTPases Rab1a, Rab1b, and Rab11a.

Conclusions: Our findings unravel a novel and previously uncharacterized role for PCa cell-derived exosomes in MET and clonal expansion of prostate tumors through neoplastic reprogramming of tumor-tropic ASCs. Targeting tumor-derived exosomes may represent a new therapeutic modality to circumvent metastatic tumors in cancer patients.

This work was partially supported by grants from the NIH/NCI (U01CA149204-01A1; A.B.A), ACS (RSGT-09-248-01-CCE; A.B.A), and two DoD grants (PC102056; A.B.A. and PC080811, D.M.).
PROTEOMIC NETWORK ANALYSIS OF BONE MINERAL DENSITY VARIATION AT DIFFERENT MENOPAUSE STATUS IN CAUCASIAN FEMALES.

Lan Zhang

Center for Bioinformatics and Genomics, Department of Biostatistics and Bioinformatics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA.

Menopause occurs usually during women’s late 40s or early 50s, and it is one of the crucial physiological events in the female life. The status change of menopause is accompanied by extreme fluctuations in hormone levels and can increase risk for numerous health problems. Peripheral blood monocytes (PBM), well known as the potential osteoclast precursors, can differentiate into various cell types that are close related to immune response and tissue morphogenesis. Using the quantitative proteomics methodology LC-nano-ESI-MS\textsuperscript{5}, we performed protein expression profiles of PBM in healthy pre-and postmenopausal women. Proteins encoded by 64 genes are significantly differential expressed, and the regulation trends of 15 genes are confirmed by the corresponding microarray study. Gene Ontology (GO) terms were identified for these differentially expressed genes, and major KEGG pathways were found relevant to biological processes as cellular metabolism, cell adhesion, immune disease and the others. To study network patterns in the detected expression changes, we also applied Gene Set Enrichment Analysis (GSEA) between pre and post-menopausal group as well as low and high-BMD group. The results of GSEA revealed some complementary evidence about possible metabolic mechanism to our study.
Citrate is an important inhibitor of calcium nephrolithiasis and its urinary excretion has been thought to be controlled by NaDC1 on the apical membrane of the proximal tubule. However, we have recently identified a novel citrate transport system and also have found citrate transport in proximal tubule cells grown from NaDC1 knockout mice. To further define the molecular details of citrate transport regulation, we have used established cell lines from S1, S2 and S3 segments of the proximal tubule and the mouse proximal tubule cell line BUMPT-306, which transport citrate. However, the expression of citrate transport proteins and related regulatory proteins in these cell lines has not been characterized.

The present studies address the expression profiles of various citrate and dicarboxylate transporters and associated regulatory proteins in these cells. NaDC1 exhibited expression in BUMPT > S1 >> S2, S3 (P < 0.05); in contrast, the presumed basolateral transporter NaDC3 exhibited expression in S3 > S1 > BUMPT, S2 (P < 0.05). Other transporters that may be involved in citrate transport or its regulation (PAT1, DRA, NaCT) exhibited different patterns of expression that diverged from either NaDC1 or NaDC3.

To characterize the adaptation of expression of these gene products to acidosis, we studied mRNA expression in vivo in response to acidosis, in both NaDC1 heterozygous and knockout mice. In NaDC1 knockout and heterozygous mice, acidosis increased the expression of NaDC3, PAT1 and DRA. In NaDC1 heterozygous mice, acidosis also significantly increased NaDC1, NaCT, Megalin, and GGT expression levels.

In conclusion, proximal tubule cell lines express mRNA for various dicarboxylate transporters and regulatory proteins in varying patterns. However, there is no strict correlation with the in vivo patterns of expression. In vivo, acidosis induces expression of a variety of citrate and dicarboxylate transporters.

This work was supported by NIH/NIDDK.
BLOOD PRESSURE RESPONSE TO THE COLD PRESSOR TEST PREDICTS THE RISK OF HYPERTENSION

Zhao Q*, He J*

*Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA

Hypertension has become an enormous global public health burden because of its high prevalence and related risk for cardiovascular disease and premature death globally. Early detection of its signs and risk factors is crucial for its prevention. Blood pressure (BP) hyperreactivity to the cold pressor test (CPT) has been suggested as a predictor of hypertension. We examined whether BP responses to the CPT predicted the risk of hypertension in the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study participants from north rural China. A total of 1,961 participants without any antihypertensive treatment completed the CPT at the baseline examination. Hypertension status was assessed by nine BP measurements obtained during 3 consecutive days at baseline (2003-2005) and two follow-up visits (2008-2009 and 2011-2012). After adjustment for multiple covariates, both systolic and diastolic BP responses to the CPT were significantly associated with hypertension incidence. For example, greater maximum response of systolic and diastolic BP during the CPT were both significantly associated with increased hypertension incidence (\( P = 0.008 \) and \( 0.0007 \), respectively). Compared with the lowest quartile of the maximum systolic BP response, the odds ratios (95% CI) for developing hypertension were 0.92 (0.66, 1.39), 1.42 (1.03, 1.97) and 1.45 (1.05, 2.00) for the second, third, and fourth quartiles, respectively (\( P \) for trend = 0.004). In addition, the effects of BP response to the CPT on the development of hypertension did not vary by age. However, interaction analyses of BP response with sex and baseline BP levels showed that the effect of maximum systolic BP response on incident hypertension was more manifest among women and subjects with lower BP levels at baseline (both \( P \) for interaction = 0.02). Hyperreactors (defined by the maximum SBP response to the CPT) exhibited greater risk for developing hypertension than normoreactors in women (OR [95% CI] = 1.76 [1.23, 2.53] and \( P = 0.002 \)), but not in men. In conclusion, BP hyperreactivity to the cold stimulus may predict the risk of hypertension in the Chinese population. The CPT may be a useful predictor for hypertension from young adulthood to old age. In addition, certain response measurements during the CPT may be more effective in the risk prediction of hypertension among women and individuals with lower BP levels. Furthermore, future studies in elucidating the mechanisms underlying the association are necessary to determine the causality of BP hyperreactivity with the development of hypertension.

The GenSalt study is supported by a research grant (R01 HL087263) from the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. Dr. Qi Zhao is supported by Award K12HD043451 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institute of Health.
IDENTIFICATION OF PROTEINS IMPORTANT FOR MALE OSTEOPOROSIS FROM HUMAN PERIPHERAL BLOOD MONOCYTES

Authors: Yingchun Zhao, Tulane University, USA, Lan Zhang, Tulane University, USA, Hualin Huang, Tulane University, USA, Yao-Zhong Liu, Tulane University, USA, Yong-Jun Liu, Tulane University, USA, Fei-Yan Deng, Tulane University, USA, Hong-Wen Deng, Tulane University, USA

Introduction: Osteoporosis is a major public health problem, mainly characterized by low bone mineral density (BMD), which is largely genetically determined with gender-specificity. Although women generally have lower BMD than men, men suffer significantly higher mortality rate upon osteoporotic fractures. However, current studies have been largely focused on female osteoporosis. Low BMD may result from bone resorption (by osteoclasts) exceeding bone formation (by osteoblasts). Peripheral blood monocytes (PBMs) are precursor cells for osteoclasts. We are utilizing PBMs, which are directly isolated from male subjects with high or low BMD, for protein profiling with subcellular extraction and 2D-nanoLC-ESI-MS/MSE for the purpose of identifying proteins important for male osteoporosis.

Method: PBMs are isolated from the whole blood with the negative immunomagnetic separation method. We used ProteoExtract Subcellular Proteome Extraction kit to isolate proteins of membrane, cytosolic, nuclear, and cytoskeletal fractions. After trypsin digestion, the peptide mixture is separated with 2-dimensional NanoAquity System. The five fractions generated from the high pH first dimension LC are further separated with the reverse phase second dimension LC. The separated peptides were analyzed with Synapt G2 MSE system. Data collection was controlled by Masslynx 4.1. Protein identification and quantification were performed in PLGS 2.4. Data analysis was performed in SPSS and Excel.

Results: We have recruited and phenotyped 120 males at peak bone mass ages of 25-50 based on low and high hip BMD values. We are using half of the sample (30 low vs. 30 high BMD subjects) for protein profiling. After searching against IPI database (v3.83), a typical profiling generated 3275 proteins for cytoplasm fraction, 3203 proteins for membrane fraction, 1037 proteins for nuclear fraction, and 967 proteins for cytoskeletal fraction with a total of 8483 proteins. We identified 33 upregulated and 3 downregulated proteins (low BMD vs high BMD) in cytosolic component; 87 upregulated and 17 downregulated proteins in membrane component; 4 upregulated and 3 downregulated proteins in nuclear component; and 39 upregulated and 30 downregulated proteins in cytoskeletal component. Western blot validation of selected significant proteins is being carried out. Functional study will be pursued for those validated significant proteins.
Novel Metabolic Markers for the Risk of Carotid Plaque Progression in American Indians

Yun Zhu,¹ Noorie Hyun,² Donglin Zeng,² Karan Uppal,³ ViLinh T. Tran,³ Tianwei Y,⁴ Dean Jones,³ Jiang He,¹ Elisa T. Lee,⁵ Barbara V. Howard,⁶ Jinying Zhao¹

¹Department of Epidemiology, Tulane University School of Public Health, New Orleans, LA
²Department of Biostatistics, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC
³Division of Pulmonary, Emory University School of Medicine, Atlanta, GA
⁴Department of Biostatistics and Bioinformatics, Emory University School of Public Health, Atlanta, GA
⁵Center for American Indian Health Research, University of Oklahoma Health Sciences Center, Oklahoma City, OK
⁶Medstar Health Research Institute and Georgetown and Howard Universities Centers for Translational Sciences, Washington, DC
**Background:** Coronary artery disease (CAD) is the leading cause of death among all racial and ethnic groups in the United States. American Indians have been affected with disproportionately high rates of CAD and related metabolic disorders. CAD is a metabolic disorder characterized by atherosclerosis in the epicardial coronary arteries. Carotid atherosclerotic plaque is the hallmark of CAD. Despite substantial research, the mechanisms underlying CAD remain elusive. Although traditional coronary risk factors such as age, gender, obesity, smoking, hypertension, diabetes and dyslipidemia account for a considerable proportion of disease variability, they have limited value in early prediction of disease risk. Metabolomics is an emerging high-throughput analytical technology that can simultaneously quantify hundreds to thousands of metabolites in biofluids, and thus provides a powerful tool for early biomarker identification of metabolic disorders. Previous metabolomics studies on CAD were primarily cross-sectional using a targeted metabolomics approach by focusing on a subset of pre-selected metabolites. Moreover, almost all existing metabolomics studies on CAD were conducted in European populations, which may be quite different from that of other ethnic groups. Using an untargeted high-resolution metabolomics approach, the current study seeks to identify novel metabolic predictive markers of carotid plaque progression in American Indians in the Strong Heart Study, a large prospective cohort study of American Indians residing in Oklahoma, Arizona and South/North Dakota.

**Methods:** This analysis included 396 apparently healthy American Indians who were free of overt cardiovascular disease (CVD) and type 2 diabetes at baseline (2001-2003) and followed through the end of 2006-2009. Carotid plaque was assessed by ultrasound at both clinical visits. Carotid plaque progression was defined as having a higher plaque score at the end of study follow-up compared to baseline. Relative abundance of 1,364 metabolites matching in the current metabolomics databases (HMDB, Lipid Maps and Metlin) in fasting plasma was detected using an untargeted metabolomics approach via high resolution LC-MS. The association of carotid plaque progression with each metabolite was examined using a
parametric proportional hazard model with a Weibull-distributed baseline hazard function adjusting for traditional coronary risk factors, including age, gender, study center, body mass index, low density lipoprotein, high density lipoprotein, systolic blood pressure, smoking and drinking status and glomerular filtration rate. To examine the combined effects of metabolites on carotid plaque risk, a multi-marker metabolites score was constructed based on the sum of significant metabolites concentration weighted by their regression coefficient. We calculated the net reclassification improvement index (NRI) to evaluate the incremental value of the metabolic markers for risk prediction by comparing the logistic regression model with traditional clinical risk factors only and the model with both clinical risk factors and the detected metabolites. Sparse partial least square discriminant analysis (sPLS-DA) was used to access the stratification of participant with/without plaque progression using the significant metabolites. Multiple testing was corrected using the q-value method (q-value <0.05 was considered statistical significance).

Results: Among the 396 participants, 100 individuals exhibited carotid plaque progression. Participants with plaque progression were relatively older, had higher levels of low density lipoprotein and systolic blood pressure, but lower level of glomerular filtration rate. There are no significance difference in BMI and HDL were observed between participants with or without plaque progression. After adjusting for clinical covariates, 6 metabolites in the classes of glycerophosphocholines, sterols, flavonoids, fatty amides and fatty acids were significantly and independently associated with carotid plaque progression. Specially, per standard deviation increase in the log-transformed levels of matching PC(18:0/18:1(11Z)) was significantly associated 38% reduced risk of plaque progression. By contrast, per standard deviation increase in log-transformed levels of matching PC(6:2/14:2), proscillaridin A, 8E-heptadecenedioic acid, flavone and N-palmitoyl methionine were associated with 56%, 68%, 56%, 69% and 72%, respectively, increased risk of plaque progression. Additional inclusion of the multi-marker score comprising of 6 metabolites significantly improves risk prediction (NRI 0.2238 [95% CI, 0.1824-0.2692]; P=1.23x10⁻⁵). Participants with carotid plaque progression and
those without can be clearly separated into two distinct groups using the 6 significant metabolites.

**Conclusions:** This is the first study to provide evidence that altered plasma metabolites in the classes of lipids significantly and independently predict risk of carotid plaque progression over and above traditional coronary risk factors. These newly detected metabolites may reveal previously undescribed new roles of various lipid species in the developing of plaque progression and could be used as novel metabolic markers for risk prediction and stratification.
C/EBPβ recruitment of TAMs is critical for preneoplastic progression

Zwezdaryk KJ¹, Coffelt SB², Medina D³, Machado HL¹

¹Department of Biochemistry and Molecular Biology, Tumor Biology and Signaling Tulane Cancer Center, Tulane University School of Medicine, New Orleans, LA
²Division of Immunology, Netherlands Cancer Institute, Amsterdam, The Netherlands
³Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX

Inflammation and the recruitment of tumor-associated macrophages (TAMs) promote tumor progression and are correlated with metastasis and poor prognosis. The events initiating a pro-inflammatory environment that aid in preneoplastic progression are poorly defined. The transcription factor CCAAT/enhancer-binding protein β (C/EBPβ) is known to activate numerous inflammatory molecules ascribed to breast cancer metastasis and poor prognosis. To determine the role of C/EBPβ transcriptional activation in recruiting TAMs during preneoplastic progression, we employed a syngeneic p53−/− mouse model, to evaluate changes in inflammation. Microarray data of two established p53+/− transplantable premalignant (PN) lines revealed an increased expression of pro-inflammatory genes in PN1a (high tumor-forming potential) versus PN1b (low tumor-forming potential) lesions. Immunohistochemistry staining confirmed that PN1a lesions recruited more macrophages than PN1b lesions and macrophage numbers and CCL2 levels increased with progression. Furthermore, as measured by FACS analysis, progression leading to invasive cancer resulted in decreased lymphoid cell numbers and increased myeloid cell numbers. C/EBPβ-LIP – one of three C/EBPβ protein isoforms - expression decreased in PN1b lesions and dramatically increased in PN1a lesions from 8-18 weeks. The results suggest C/EBPβ may regulate progression to invasive cancer through recruitment of TAMs. Additionally the macrophages may be aiding in immunosuppression within the tumor microenvironment. A clear understanding of this interaction may lead to novel anti-cancer targets and therapies.

This work was supported by a grant from the NCI