Translational Research Day Abstracts
January 26, 2018
8:30am to 12:30pm
Drug Discovery Auditorium & Lobby
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The modulation EphA2-induced cytoskeleton re-organization: a new insight into the role of extracellular Hsp90

Daoud Abdelkader, PhD, Postdoctoral Scholar, College of Medicine, Pharmacology

Category: Other

Background: The Eph tyrosine kinase receptor A2 (EphA2) has emerged as key participants in the progression of a wide range of malignancies. EphA2 modulates the cytoskeletal dynamics to control cancer cell motility and invasion. We previously showed that extracellular Hsp90 (eHsp90) exhibits functional collaboration with EphA2 independently of its cognate ligand ephrin A1. In the current study, we aimed to further define the molecular and functional relationship between EphA2 and eHsp90 within the context of ligand activation.

Results: It is known that ephrinA1 signaling promotes RhoA activation and altered cell morphology to favor transient cell rounding, retraction, and diminished adhesion. Our findings reveal that eHsp90 neutralization via either blocking antibodies or cell-impermeable Hsp90-targeted inhibitors significantly attenuated ligand dependent cell rounding in diverse cancer models including breast, prostate, melanoma and GBM, indicating a conserved mode of action. Moreover, we found that eHsp90 elicited Src/RhoA activation and enhanced ligand dependent cell rounding, retraction, and ECM detachment. Conversely, eHsp90 blockade impaired ephrin A1-mediated Src activation and formation of an EphA2-Src complex. Further, eHsp90 signaling via this axis stimulated activation of the myosin pathway, culminating in formation of an EphA2-myosin complex central for cytoskeletal remodeling. Inhibition of either eHsp90 or Src was sufficient to impair ephrinA1 mediated activation of myosin, and EphA2-myosin complex formation.

Summary and Conclusions: Collectively, our data support a paradigm whereby eHsp90 and EphA2 exhibit molecular crosstalk and functional cooperation within a ligand dependent context to orchestrate cytoskeletal events controlling cell morphology and attachment.
**M10, a 10 Amino Acid Peptide, Regulates the Extracellular Matrix Expression via a Dual Mechanism in Scleroderma Associated Lung Fibrosis**

**Tanjina Akter, MS, Graduate Student**, Atanelishvili I, Silver RM, and Bogatkevich GS

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**Background.** Interstitial lung disease (ILD) is the major cause of mortality among scleroderma (systemic sclerosis, SSc) patients. Extracellular matrix (ECM) deposition is a hallmark of this and other fibrotic lung diseases. TGFβ regulates ECM gene expression, Ca2+ level, matrix metalloproteinase (MMPs), and tissue inhibitors of metalloproteinase (TIMPs). We recently demonstrated that M10 peptide, naturally derived from the hepatocyte growth factor receptor, blocks the TGFβ-mediated canonical pathway and reduces fibrosis in vivo. In this study, we investigate the efficacy of M10 peptide in the regulation of intracellular Ca2+ levels, ECM genes [collagen I, connective tissue growth factor (CTGF), fibronectin and tenascin] transcriptions, and MMPs/TIMPs expression in primary lung fibroblasts.

**Results Summary.** In both normal and SSc-ILD lung fibroblasts, TGFβ induced an acute intracellular Ca2+ efflux at 15sec, a delayed efflux at 60sec and maintained a high level of Ca2+ throughout the cellular screening process. In the presence of M10 peptide, TGFβ-mediated Ca2+ was significantly (p < 0.001) reduced in both acute and delayed states maintaining overall lower amplitude. M10 peptide suppressed TGFβ-mediated ECM genes transcriptions. TGFβ significantly induced MMP-3 expression and suppressed TIMP-2, while M10 peptide blocked TGFβ-mediated TIMP-2 inhibition and restored it to normal levels. M10 peptide significantly induced MMP-10 in normal lung fibroblasts but not in SSc-ILD fibroblasts.

**Conclusions.** M10 peptide reduces mRNA of ECM proteins, inhibits TGFβ-mediated Ca2+ efflux, regulates MMPs and TIMPs targeting degradation of collagen and other ECM proteins. These data suggest that M10 peptide possesses great potential to reduce TGFβ-mediated outcomes in SSc-ILD.
Alexander Alekseyenko, PhD, Associate Professor, College of Medicine, Biomedical Informatics Center

Category: Other

PESAME: Predictive effect size analysis in multivariate ensembles

Background
Biomedical research generates troves of high dimensional biological measurements, such as gene expression, protein abundances, microbial compositions, etc. A common analysis task with such data is to determine the performance of the individual predictors from these datasets in predicting one or several relevant response variables, such as disease status or survival. Common screening or feature selection methods may use parametric or non-parametric univariate testing. Mann-Whitney test and its underlying U-statistic is interesting for this task because of their direct connection to a robust measure of predictivity, the area under receiver operating characteristic curve (AUC).

Results Summary
We developed an R Shiny application, predictive effect size analysis in multivariate ensembles, that allows for screening of high-dimensional predictor collections in their predictive signal for one or more responses. The application allows to compute the Mann-Whitney test significance, estimate AUC and its error bounds, and to control for multiple comparisons using several popular techniques. The user is able to perform rudimentary processing of the response variables to define the exact analysis end points. The application produces results in tabular and graphical formats of publication quality.

Conclusions
In designing and implementing the application, we have determined essential components of a robust user interface that will enable development of similar applications in the future. Using the lessons learned from this exercise, we will continue to develop a suite of tools that (1) do one thing only and do it well and that (2) work well together.
Parent Preferences for Methods and Content of Technology Based Asthma Risk Communication

Annie Andrews, MD, MSCR, Associate Professor, Medicine, Pediatrics

Category: KL2 Scholar

Background/Objective: Interventions to improve care for children with asthma increasingly utilize mobile technology. Little is known about parent preferences for mobile technology based asthma interventions. Our objective was to develop insight into parent use of mobile technology and their preferences for content of an asthma risk communication intervention.

Methods: Interviews of parents of children with asthma were conducted. The open-ended, semi-structured interview guide included questions about current mobile technology use, barriers to controller medication adherence, and preferences for methods and content of a mobile technology based asthma risk communication intervention. Using grounded theory methodology, investigators coded the transcripts and identified emerging themes.

Results: 20 parents of children with persistent asthma completed interviews. 80% of the children had public insurance. 40% had a history of ICU admission for their asthma. 35% were currently on a combined ICS/LABA. Three major themes were identified: Room for improvement/welcoming help, distinct preferences for risk communication, and electronic reachability. Room for improvement/welcoming help includes caretakers recognizing that busy lifestyles contribute to adherence challenges and all participants welcomed an electronic solution that could help them care for their child’s asthma. Distinct preferences for risk communication includes a preference for two-way communication with a clinical provider, phone call or text message and monthly contact. Electronic reachability includes parents’ electronic habits.

Conclusions: Parents of children with asthma are open to communicating with asthma providers through mobile technology. This information can be used to inform the development of future mobile technology based interventions to improve care for children with asthma.
Functional Characterization of Mutant BRCA1

John Barrows, BS, Graduate Student, Biochemistry & Molecular Biology

Category: TL1 Trainee

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BRCA1 (breast cancer susceptibility protein 1) plays a major role in preserving genome integrity through its involvement in various aspects of the DNA damage response. BRCA1 mutations are the primary cause of hereditary breast and ovarian cancers, with patients exhibiting ~80% lifetime risk of developing these cancers. In addition, many sporadic breast tumors lack functional BRCA1, further implicating BRCA1 dysfunction in tumorigenesis. Clinical mutations in BRCA1 cluster within three highly conserved functional domains: the N-terminal RING domain, a central coiled-coil domain, and a C-terminal tandem BRCT domain. However, the role that each BRCA1 functional domain plays in DNA repair and tumor suppression remains unclear. By developing a new method to isolate intact BRCA1-mutant complexes, we will identify the molecular consequences of specific BRCA1 clinical mutations. These studies will elucidate how BRCA1 defects lead to cancer and support the development of new therapeutic strategies for different BRCA1-mutant tumors.

This project is supported by the South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, through NIH Grant Numbers TL1 TR001451 and UL1 TR001450, as well as through the NIH (R35GM119512)
Wall Tension Regulates microRNA-133a in Thoracic Aortic Aneurysm Development

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Category: Other

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Background:

Loss of cellular microRNA-133a is associated with thoracic aortic dilation in aneurysm. According to the Law of Laplace, wall tension increases with increasing vessel diameter. This study hypothesized that increased wall tension accounts for reduction in cellular miR-133a in thoracic aortic aneurysm.

Results Summary:

Tension reduced microRNA-133a in ex vivo applications. Tension reduced microRNA-133a in aortic fibroblasts but not smooth muscle cells in in vivo studies. Tension did not affect transcription of microRNA-133a. Additionally, tension also did not affect transcription or protein abundance of microRNA targeting exoribonucleases. Tension did increase exosome secretion from fibroblasts as well as microRNA-133a levels in those exosomes. Pharmacologic attenuation of exosome release inhibited the loss of cellular microRNA-133a in response to increased tension. Two mouse models of increased wall tension showed increased microRNA-133a levels in the plasma. Normalization of microRNA-133a levels by a lentiviral vector attenuated aneurysm formation in a murine model.

Conclusions:

Exosome release by fibroblasts in response to tension represents a probable mechanism for loss of microRNA-133a in thoracic aortic aneurysm. Inhibition of this loss offers a novel target for therapeutics.
Multivariate Air Pollutant Exposure Prediction in South Carolina

Ray Boaz, Department of Public Health Sciences, College of Graduate Studies, Medical University of South Carolina

Category: TL1 Trainee

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Introduction:

Air pollution is associated with adverse health outcomes ranging from increased respiratory incidence to increased mortality; however, the health impacts from exposure to multiple pollutants remain unclear. Large gaps in knowledge remain for developing flexible models that address the decomposition of chemical mixtures in relation to health outcomes. In particular, application of complex fusion models, which combine observed and modeled data, to areas with sparse monitoring networks with multiple chemical components is under-developed. Such models could provide improved accuracy and coverage for air quality measurement predictions, an area greatly limited by the amount of missing data.

Methods:

This project focuses on the development of methods for improved estimation of pollutant concentrations when only sparse monitor networks are found. Sparse monitoring networks are defined as areas where fewer than three criteria air pollutants (based on EPA standards) are monitored. Particularly, a multivariate air pollutant statistical model to predict spatio-temporally resolved concentration fields for multiple pollutants simultaneously is developed and evaluated. The multivariate predictions allow monitored pollutants to inform the prediction of non-monitored pollutants in sparse networks.

Results/Impact:

These methods utilize only widely available data resources, meaning that the improved predictive accuracy of sparsely monitored pollutant concentrations can benefit future studies in any US area by improving estimation of health effects and saving resources needed for supplemental air pollutant monitoring campaigns. Our method for estimation attempts to improve predictive accuracy and data availability for sparsely monitored pollutants and areas.

Grant Acknowledgement: TL1 TR001451 & UL1 TR001450
The cell-cell adhesion component PLEKHA7 regulates the pro-tumorigenic MIR17HG long non-coding RNA in colon epithelial cells

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Category: TL1 Trainee

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Background:

The adherens junctions (AJs) are essential architectural elements of epithelial tissues. Recently, we identified a novel mechanism whereby the AJs suppress aberrant colon epithelial cell behavior by recruiting the RNAi machinery, mRNAs, and miRNAs, via the AJs component PLEKHA7. Our data reveal dysregulation of PLEKHA7 and this mechanism in colon cancer cell lines and patient tissues. Interestingly, RNA-CLIP/RNA-Seq identified association of PLEKHA7 with numerous long non-coding RNAs (lncRNAs). LncRNAs interact with the RNAi machinery in multiple ways. While lncRNAs have been associated with tumorigenesis, the mechanisms of their regulation are unclear. We hypothesize that the AJs regulate the levels of lncRNAs via PLEKHA7 and junctional RNAi machinery to suppress pro-tumorigenic colon cell behavior.

Results:

RNA-Seq identified junction-associated lncRNAs whose expression levels are regulated by PLEKHA7. The top upregulated lncRNA upon PLEKHA7 depletion was MIR17HG, an oncogenic host transcript of a cluster of miRNAs. These mature miRNAs also co-precipitate with PLEKHA7. PLEKHA7 knockdown results in increased levels of MIR17HG, but only a subset of its hosted miRNAs (miR-19a,b). Notably, miR-19a and mir-19b are highly upregulated in colon cancer. Our data suggest that two PLEKHA7-associated miRNAs, miR-203a and miR-372, mediate suppression MIR17HG. Re-expression of PLEKHA7 in aggressive colon cancer cells that lack PLEKHA7 suppressed expression of MIR17HG, as well as anchorage independent growth of these cells.

Conclusion:

Our data point towards a novel mechanism of lncRNA regulation that tethers epithelial tissue integrity with pro-tumorigenic cell transformation. Reducing elevated MIR17HG levels, is a potential therapeutic approach to suppress the tumorigenic behavior of cells that have lost their junctional integrity and homeostasis.

The project described was supported by the NIH National Center for Advancing Translational Sciences (NCATS) through Grant Numbers TL1 TR001451 & UL1 TR001450.
Cortisol Levels and Situational Use Factors in Cocaine-Dependent Women

Lauren Campbell, Student, College of Charleston, MUSC Employee, Psychology and Addiction Sciences

Category: Other

Background. Biological and situational stressors play important roles in cocaine relapse risk, yet there has been little investigation into their association. The current study extended previous research which found gender-specific susceptibility to cocaine craving and relapse under stressful conditions, by investigating the association between a measure of drug taking situations and cortisol levels in cocaine-dependent women during an in-vivo cocaine-cue stress test preceded by a pharmacological stressor.

Method. In this double-blind, placebo controlled study, non-treatment seeking cocaine-dependent women (N=23) were administered yohimbine hydrochloride (21.6 mg) or placebo in a counterbalanced fashion prior to two cue reactivity stress procedures. The Inventory of Drug Taking Situations (IDTS), administered at baseline, is a 50-item self-report survey measuring antecedents to relapse. Cortisol levels were measured before and at the start of the cocaine cue stress test, and before and after yohimbine (or placebo) administration. We hypothesized that cocaine-dependent females with higher IDTS scores would have a greater cortisol response.

Results. Cortisol levels after yohimbine administration and before the cocaine-cue stress test were positively correlated with the negative (p<.05) and positive (p<.01) IDTS subscales. Cortisol levels during the cocaine-cue stress test were positively correlated with the negative (p<.01), positive (p<.01), and temptation (p<.05) subscales.

Conclusion. These results may be useful in treatment settings where mindfulness about situational triggers can be used to identify physiological reactivity, patterns of use, and subsequent relapse. Future research might focus on other related physiological measures to see if they correlate similarly to IDTS scores in other populations.
Fibroblasts are mesenchymal cells that release collagens, laminins, and proteoglycans, which are essential for functional connective tissue. Myofibroblasts are activated-fibroblasts that express α-smooth muscle actin and contract during wound healing. If activation continues, myofibroblasts may become fibrotic resulting in fibrosis (excessive and aberrant deposition of extracellular matrix components that leads to dysregulated function in the target organs). Despite this information, the molecular events of fibrosis are not completely understood. Previously, we show that fibroblasts express the Nα-Formyl-L-methionyl-L-phenylalanine receptor (FPR1). FPR1 is a 7TM spanning G-protein coupled receptor that regulates the leukocyte response to bacterial formylated peptides by facilitating activation of such peptides that typically also leads to leukocyte migration towards such peptides. We exposed intestinal fibroblasts to series of inflammatory mediators that include cytokines IL-6, IL-8, IL-10, interferon gamma, and bacterial formylated peptides respectively. We then examined how the mediators affect the ability of the fibroblasts to attached to fibrinogen (CD11b ligand). Interestingly, following treatment with bacterial formylated peptides (fMLP), fibroblasts adhesion to fibrinogen was increased by 50%. Moreover, treatment with a FPR1 inhibitor resulted in decreased adhesion, but gene silencing (siRNA) of the FPR1 resulted in increased adhesion. Taken together, FPR1 appears to modulate the activation of fibroblasts. Reduced activation of the FPR1 may lead to a better understanding of the fibrotic response and better treatments of dysregulated inflammatory conditions involving fibroblasts.
Pharmacological exposures effect cranial suture stem cells precipitating premature suture fusion

Emily Durham, MA, Student, Oral Health Sciences, R. Nicole Howie, Amanda LaRue, James Cray

Category: Other

Background & Purpose – The Centers for Disease Control and Prevention, National Birth Defects Study has published data suggesting that "environmental" exposures including maternal thyroid diseases, use of selective serotonin reuptake inhibitors (SSRIs) in pregnant mothers, and maternal nicotine use may exacerbate incidence and or severity of craniofacial anomalies including craniosynostosis. Craniosynostosis is a birth defect defined as the premature fusion of a suture(s) of the skull occurring in 1:1800-2500 births. A proposed mechanism of craniosynostosis is the disruption of the balance of proliferation and differentiation of the osteogenic precursors including stem cells in the perisutural area leading to bone overgrowth within cranial sutures. Here, we hypothesize that the teratogenic exposures including excess thyroid hormone, SSRI and nicotine lead to a depletion of stem cells within the suture resulting in premature suture fusion.

Results – Assessment of coronal and posterior interfrontal suture fusion revealed that in utero exposure to nicotine increased the risk of premature posterior interfrontal suture fusion. Exposure to citalopram resulted in mouse pups that were more likely to have coronal and posterior interfrontal suture fusion at post-natal day 15 as assessed by micro-CT. Further, we confirmed a reduction in Gli1+ cells ex vivo in correlation with in utero teratogen exposure. Our in vitro analysis also indicates a depletion of stem cell populations with teratogen exposure via flow cytometry.

Conclusions – Teratogenic exposures including maternal thyroid disorder, maternal use of SSRIs and maternal nicotine use may target calvarial stem cell populations for depletion precipitating an increased risk for craniosynostosis.
Characterization of Pericytes from Normal and Idiopathic Pulmonary Fibrosis (IPF) Human Lungs

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Category: Other

Background. Pericytes are key regulators of blood vessel development and function. However, pericytes may play additional roles in tissue homeostasis and repair through their ability to upregulate immune response genes and transdifferentiate into myofibroblasts. Accumulation of myofibroblasts is a hallmark of fibrotic diseases such as Idiopathic Pulmonary Fibrosis (IPF). To understand the role of pericytes in lung fibrosis, we isolated these cells from normal and IPF lungs to compare their properties and responses to fibrotic and inflammatory stimuli in vitro.

Results. Pericytes were selected from explanted human lung digests based on PDGFRβ expression as we previously described for mouse cells. We found that IPF cells migrated significantly more rapidly and invaded a matrix more readily than normal pericytes. TGFβ, a major fibrotic cytokine, caused both normal and IPF pericytes to shift to a myofibroblastic phenotype, with increased expression of collagen, αSMA, and fibronectin. Given that pericytes are uniquely positioned in vivo to respond to danger signals of both systemic and local origin, we stimulated pericytes with agonists having damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs). Both normal and IPF lung pericytes showed increased expression of proinflammatory chemokines in response to PAMPs and DAMPs.

Summary and Conclusions. Our results demonstrate that human lung pericytes can transition to myofibroblasts, and IPF pericytes are more invasive than normal. IPF and normal pericytes both respond to danger signals through elaboration of proinflammatory chemokines. Further understanding the biology of normal and IPF pericytes will assist in developing targeted therapeutics for treatment of fibrosis.
Tuning micelle composition to improve targeted delivery of chemotherapy to brain tumors

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Category: Other

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Therapeutic drug delivery across the blood-brain barrier (BBB) is not only inefficient, but also nonspecific. Nanoparticle composition plays a significant role in determining uptake efficiency and therapeutic efficacy. In order to understand the fundamental aspects of micelle assembly and dynamics, encapsulation efficiency, and therapeutic efficacy, micelle mixtures composed of different lipid constituents, namely PEG-PE-Amine, N-palmitoyl homocysteine (PHC, pH sensitive lipid breaks in endosome pH ~5.5) or D-α-tocopheryl polyethylene glycol succinate (TPGS), were investigated. Differences in the dependencies of the micelle size parameters (core radius and overall micelle radius) on the composition originated from the differing trends in aggregation number for the two micelle series. Conjugation of targeting moieties and contrast imaging agents was quantified using high-performance liquid chromatography. Preliminary cellular uptake studies via fluorescence imaging of glioblastoma cells treated with targeted and untargeted micellar particles demonstrate considerable uptake with PDGF-micellar PHC showing highest uptake as compared to PDGF-micellar TPGS. Our results show a strong correlation between the number of targeting monomers per micelle and the activity of uptake. In summary, the two micelle series showed similar uptake that was independent of the lipid structure or molecular weight yet significantly different dependencies of their aggregation number and size parameters.
Temporal lobe white matter structure and surgical outcomes in medically refractory temporal lobe epilepsy

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Category: Other

Background: Even though surgery for medial temporal lobe epilepsy (MTLE) is one of the most effective treatments in modern medicine, many patients remain with seizures after surgery, and the reasons for suboptimal outcomes are not well known. We aimed to determine whether presurgical plastic reorganization of temporal lobe structures is related to the likelihood of seizure freedom after surgery.

Results summary: We constructed the individual presurgical structural brain connectome of 50 patients with MTLE who underwent surgery for epilepsy and computed betweenness centrality (BC) (an integration measure of regional network hubness derived from graph theory), of all gray matter regions of interest (ROIs) throughout the brain. Ipsilateral temporal ROIs were entered into a discriminant analysis function and cross-validated classification outcomes by using a leave-one-out approach. We derived discriminant functions from clinical variables alone and a combination of clinical and connectome measures. Our results revealed a discriminatory function constituted by the BC of six ipsilateral temporal ROIs with classification accuracy of 90% for the original cases and 82% on cross-validation, and was especially reliable at predicting which patients would be seizure free after surgery (97.2% of the original group, 91.6% on cross-validation). In addition, the discriminatory function based on connectome measures alone was more accurate in classifying surgical outcomes than the functions based on clinical data alone (accuracy of 46%).

Conclusion: Our results indicate that increased structural integration of gray matter regions in the temporal lobe ipsilateral to seizure onset is largely associated with lower probability of seizure freedom after surgery.
The Hidden Microbiome Pipeline: Providing Access to Clinical Microbiome Specimens, Sequences, and Informatics Resources

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Category: Other

Background: Hospital acquired infections are a major concern for healthcare systems; MUSC has developed an active infection surveillance culture program to identify patients with epidemiologically significant organisms and implement practices limiting spread of infections. This program generates surplus materials, which are typically discarded but can be used for research into structure and function of hidden microbiota.

Drug-resistant infections such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococci (VRE) are associated with severe comorbidities and complications for patients. We will determine the feasibility of extracting microbiome features from excess specimens and perform high-throughput 16S rRNA sequencing and metagenomic assays. We will study the role of microbiota in infection by developing predictive models using clinical patient data microbiome composition. We will develop Living μBiome Ban, a system for just-in-time capture and collaborative sharing of human microbiome specimens and patient data for interested investigators, within and beyond MUSC. Lastly, we will test the pipeline by performing pilot studies using Living μBiome Bank and store obtained data into MUSC research data warehouse (RDW).

Results and Conclusion: By executing this project, we will demonstrate development and implementation of protocols for surplus specimen recruitment, handling, storage, processing, high-throughput metagenomics sequencing, and bioinformatics analysis. We will confirm important predictive characteristics of microbiota and convey benefits of large-scale biobanking to research organizations and investigators. The results of our project will allow a larger number of investigators to conduct high-quality translational research with specimens, considering varying levels of institutional and investigator informatics penetration and sophistication.
Single cell genomic profiling of human B cells that are responsible for immune response against pneumococcal polysaccharides in aging HIV-negative and HIV-positive individuals

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Category: TL1 Trainee

Background: The introduction of combined anti-retroviral therapy resulted in a significant improvement of life expectancy of HIV-positive individuals leading to a rapid growth of aging HIV-positive population. Together, aging and HIV infection, increase susceptibility to life-threatening infections caused by Streptococcus pneumoniae. Despite preventative strategies, such as pneumococcal vaccination, it remains a challenge to induce potent and durable immune responses against pneumococcal polysaccharides (PPS) possibly due to poorly characterized perturbations in the B cell compartment of immune system of HIV-positive persons. The goal of this study is to characterize individual cellular changes in IgM memory B cell population that is largely responsible for producing immune responses to PPS, identify variations in inter-cellular gene expression that shape polysaccharide–specific B cell responses, and determine how aging affects these gene expressions.

Methods: Blood samples were collected pre- and post-pneumococcal vaccination from healthy and HIV+ individuals ages 21-40 and 50-65 according to guidelines of institutional review board of Medical University of South Carolina and were used for flow cytometry and single-cell genomics analysis.

Results/Conclusions: Despite the fact that both aging HIV-negative and HIV+ individuals have significantly lower numbers of B cells and overlapping B cell perturbations, as compared to healthy young adults, the immune responses to pneumococcal vaccination are different. We have shown that the phenotype of polysaccharide specific B cells changes with age from predominantly IgM-memory to switched memory in HIV-negative individuals. However, in aging HIV-positive individuals, it resembles the phenotype of HIV-negative young adults in significantly reduced percentages. Single-cell genomic studies of IgM memory B cells revealed differential expression of genes that play an important role in T-cell independent immune responses (TACI, BAFF-R, CD21, TLR9, AICDA and other), B cell proliferation, and signaling between HIV-positive and HIV-negative persons in all age groups. Furthermore, unbiased clustering analysis identified distinct subgroups within IgM memory B cell population. Together, these data significantly increase our knowledge of the genetic identities of B cells in aging HIV+ individuals uncovering their complexity and diversity and revealing insights into mechanisms underlying B cell dysfunctional phenotype.

The project described was supported by the NIH National Center for Advancing Translational Sciences (NCATS) through Grant Numbers TL1 TR001451 & UL1 TR001450; RO1 AG045973
Psychometric Evaluation using Rasch Analysis of Patient-Reported Quality of Life Post-ACL Reconstruction

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Category: TL1 Trainee

Background: The Knee Injury Osteoarthritis and Outcomes Survey (KOOS) is a commonly used instrument to measure patient-reported QOL post-ACLR. The purpose is to evaluate the psychometric properties of the QOL subscale of the KOOS.

Methods: Rasch analysis of KOOS QOL subscale from 39 individuals 1-2 years post ACLR was conducted. Measurement properties and model fit of the rating scale, items, and persons were evaluated. Mean square fit statistics ≥1.6 and standardized z-scores ≥2.0 were indicative of person/item misfit. Relationship of item difficulties and person measures was evaluated using probability curves and item maps. Reliability indicators were also examined.

Results: All items demonstrated infit and outfit mean squares and standard z-scores. The majority of persons (n=38, 97.4%) demonstrated fit to the Rasch model. However, ceiling effects were noted (n=4, 10.26%), indicating some participants report higher QOL than is measurable. The mean person measure was 1.73 logits higher than the mean item measure: this sample is skewed toward higher QOL. Person reliability was adequate (0.67) and person separation was 1.42. Calculation of person strata revealed that the KOOS QOL separated participants into two strata.

Conclusions: Although all items of the KOOS QOL fit the model, not all categories of the rating scale were used. Overall, this sample reported high QOL, which is to be expected given the time since ACLR. If participants with a broader range of time since ACLR were included, that the KOOS QOL could identify additional person strata.

Keywords: ACL reconstruction, patient-reported outcomes, Rasch

This project was supported by the South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, through NIH Grant Numbers TL1 TR001451 and UL1 TR001450.
Inflammatory Mediators and Fibroblasts

La’Toya James, MD Candidate, Medicine, Regenerative Medicine & Cell Biology
and Titus A. Reaves

Category: Other

Fibroblasts are mesenchymal cells that release extracellular matrix proteins such as collagens, laminins and proteoglycans during tissue development and tissue repair (excessive repair leads tissue scarring). Fibroblasts affect neighboring cells through the release of cytokines, growth factors, and differentiation factors. Despite this information, little is understood about the fibroblast response to diseases characterized by acute inflammation. Necrotizing enterocolitis (NEC), is a devastating disease in response to microbial infiltration which mostly affects the gastrointestinal tract of premature infants. NEC results in nearly 2% of deaths of newborns annually. Survivors of NEC display excessive scarring in the intima of the intestinal epithelium and may develop strictures leading to intestinal blockage. In the current study, we examined the fibroblast response following exposure to the following inflammatory cytokines: interferon gamma (INF-γ), interleukin-6, interleukin-8 and the anti-inflammatory cytokine, interleukin-10. Additional inflammatory mediators Nα-Formyl-L-methionyl-phenyalanine (fMLP), Lipopolysaccharides ((LPS), 4524 [E. coli], 2515 [E. coli], Salmonella minnesota) were also exposed to fibroblasts. Fibroblast morphology shows that 4524 resulted in substantial growth with cells displaying a spindle shape while exposure to INF-γ resulted in reduced growth with cells displaying less of the spindle morphology, but with enlarged nuclei. Other inflammatory mediators displayed minimal differences. We examined CD36 in fibroblasts exposed to 4524 and INF-γ. Expression was reduced in INF-γ-exposed cells suggesting that CD36 may be involved in cellular over-growth of fibroblasts exposed to LPS. Taken together, excessive growth and activation of fibroblasts may contribute to acute inflammatory conditions like NEC.
Community Hospital Factors Associated with Adoption and Implementation of a University-Based ICU Quality Improvement (QI) Outreach Program

Johnson, Emily, PhD, Assistant Professor, Nursing, Sterba, K., Warr, E., Beeks, R., Zapka, J., Ford, D.

Category: Other

Background: Adoption and implementation of evidence-based practice is challenging. A recent four-phase implementation model identifies outer and inner contextual factors related to the exploration, adoption, implementation, and sustainment of change phases. Within each phase, it is essential to identify barriers and facilitators to adoption and implementation processes in changing evidence-based practices. In this study, we identified key outer and inner contextual factors associated with adoption and implementation of an interprofessional ICU QI program, called ICU Innovations, provided by a team from the Medical University of South Carolina (MUSC) to community hospitals in South Carolina. Mixed methods research was utilized to compare attributes and processes in two hospitals with significantly different adoption and implementation processes and outcomes associated with ICU Innovations.

Results: Both inner and outer contextual factors, based on the existing four-phase implementation model, deeply influenced adoption and implementation processes associated with ICU Innovations. Primary modifiable inner contextual factors found to be most influential were communication, hospital structure, and leadership. Primary outer contextual measures found to be both modifiable and influential were relationships between partner organizations, depth of academic resource leveraging, and hospital system-level influences, such as hospital infrastructure and staffing patterns.

Conclusions: Identifying modifiable inner and outer contextual factors for ICU QI initiatives can guide needed program modifications to successfully improve adoption and implementation outcomes. Further research is needed to advance methods of contextual factor identification in the field of QI adoption and implementation, specifically in the area of community hospital based practice change.
Improvement of heart function in fibrosis models by a caveolin-1 surrogate peptide

Panneerselvem Chinnakkannu*, Charles Reese*, Dorea Pleasant-Jenkins, Elena Tourkina, Stanley Hoffman, and Dhandapani Kuppuswamy, PhD, Associate Professor, Medicine, Cardiac Fibrosis.

Category: Other

Background: Chronic pressure overload (PO) leads to ventricular hypertrophy and myocardial fibrosis and results in congestive heart failure. Because caveolin-1 has been identified as a target for anti-fibrotic therapies, we previously demonstrated that the caveolin-1 scaffolding domain peptide (CSD, a 20-amino acid segment of caveolin-1 that acts as a functional surrogate) can reverse alterations in fibrosis, signaling, and heart function in the TAC model. Here, we used an Angiotensin-II (Ang-II) infusion mouse model to further explore the therapeutic benefits of CSD in heart fibrosis.

Results: Two week infusion of Ang II resulted in heart fibrosis. As in the TAC model, increases in heart fibrosis and in signaling mechanisms associated with fibrosis were reversed by daily injections of CSD. Most striking was echo data showing that increases in LV mass and posterior wall thickness in Ang-II treated mice were significantly reversed by CSD. Also, Ang-II treatment caused diastolic dysfunction as measured by tissue Doppler in that a significant increase in the isovolumic relaxation time (IVRT) was observed in Ang-II treated mice and was reversed in CSD treated mice.

Summary and Conclusions: Similar to our findings with TAC model, Ang II-induced PO causes cardiac fibrosis, increases wall thickness, and compromises cardiac function. CSD treatment reverses these changes. The recruitment of monocytes, their differentiation into fibroblasts, and the overexpression of Col I by these cells are sensitive to inhibition by the CSD peptide. Therefore, our studies set the stage for developing CSD as a treatment for myocardial fibrosis.
Impairments in Cognitive and Emotional Processes in Mental Illness: Transdiagnostic Meta-analyses of Neurocircuit Structure and Function

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Category: Other

Background: In a recent transdiagnostic meta-analysis of regional gray matter volume, reductions were observed across patient groups in nodes of the salience network (i.e., bilateral insula and dorsal anterior cingulate cortex (Goodkind et al., 2015). In a follow up functional neuroimaging meta-analysis of cognitive control tasks (McTeague et al., 2017) we observed transdiagnostic deficits overlapping with regions prone to gray matter loss in conjunction with a broader fronto-parietal network impairment. In the current study, we investigated these patterns during emotional processing in relation to findings from the prior meta-analyses.

Methods: Articles on functional neuroimaging of emotional processing (n=296) including patients across a range of Axis I diagnoses (bipolar, depression, anxiety, substance use, schizophrenia, psychosis) and control participants were submitted to meta-analysis with the revised Activation Likelihood Estimation algorithm (Eickhoff et al., 2009).

Results: The anterior-cingulo-insular or “salience network” in conjunction with limbic regions showed aberrations across disorders during emotional processing. Convergent functional disruption across 579 studies of 21,860 individuals completing emotional and cognitive tasks was evident in limbic and paralimbic regions including the salience network, as well as lateral prefrontal regions. Overlap of functional disruptions with prior findings of transdiagnostic gray matter reduction was evident in dorsal anterior cingulate and bilateral anterior insula/ventrolateral prefrontal cortex.

Summary & Conclusions: Targeting these overlapping regions prone to structural disruption and cognitive-emotional dysfunction may be an especially productive means of remediating functional impairment across disorders.
Integration of an HIV Patient Reported Outcome Tool in the Electronic Medical Record

Eric G. Meissner, MD, PhD, Assistant Professor, Medicine, Bryan N. Rogers, Lucas Moreira, John Gnann, Cassandra D. Salgado, Melissa L. Habrat

Category: SCTR Pilot Project

Background: Assessing medication adherence, depression, and alcohol use are important components of delivering effective care for persons living with HIV infection. Patient reported outcome (PRO) tools can facilitate communication between patients and providers and identify areas for intervention that may not arise in the context of a routine clinical visit.

Methods: We developed a focused PRO tool within the electronic health record (EHR) that includes questions about HIV medication adherence, a depression screen (PHQ-8), and an alcohol abuse screen (AUDIT-C). At the time of rooming, patients use a secure touch screen on an EHR connected tablet or on a desktop computer using a mouse. Patients enrolled in MyChart can complete PRO assessments within a week of their appointment. Scores are immediately available for clinicians to review in the EHR communication inbox and discuss with the patient during the visit.

Results: Scores can be graphed longitudinally in the results review flowsheet and can be viewed by social workers and case managers providing services for patients. Use of a smart-phrase allows immediate inclusion of PRO answers into the physician’s clinical documentation. Thus far PROs have been completed by 29 patients. Multiple patients reported missing at least 1 day of medication within the last 2 weeks (n=9) and/or missing more than 3 days of medication since their last visit (n=7, with 3 patients missing more than 10 doses). PHQ-8 scores suggested 6/29 patients met criteria for major or severe major depression while 5/29 patients had higher risk scores for alcohol abuse on the AUDIT-C.

Conclusions: Implementation of a PRO tool that interfaces with the EHR represents an important tool to facilitate patient-provider communication and assess issues germane to delivering care for patients living with HIV infection.
Probing Cognitive Control Neurocircuits: A Concurrent TMS-fMRI Investigation of State Dependence

Mithoefer OJ, BS, Student, Medicine, Psychiatry & Behavioral Sciences, Lopez JW, Dowdle LT, Badran BW, Summers PM, George MS, McTeague LM

Category: Other

Background: Transcranial magnetic stimulation delivered concurrently with functional magnetic resonance imaging (TMS-fMRI) extends conventional correlational imaging to causal neurocircuit mapping. That is, single pulses of TMS can be delivered to superficial cortical regions, and the activity in the connected networks mapped with the BOLD response. In the current study, we investigated whether state dependence, particularly emotional arousal, would influence the responsiveness of the fronto-parietal network to single pulse TMS (spTMS).

Methods: Twenty-four healthy individuals completed a picture-viewing paradigm in the MRI scanner. Pleasant, neutral, and unpleasant pictures from the International Affective Picture System were presented in blocks. While pictures were presented in the foreground, spTMS was delivered intermittently to left dorsolateral prefrontal cortex, the typical therapeutic target.

Results: Emotional picture processing increased BOLD responses to spTMS in bilateral fronto-parietal regions (i.e., left and right dLPC and intraparietal sulci). Further, strong modulation of visual processing networks as a function of emotional arousal suggests that concurrent TMS did not disrupt processing of the foreground task.

Summary & Conclusions: Therapeutic rTMS is moving toward manipulating the state-dependence of the patient during treatment delivery. This includes clinically-relevant immersive environments, visual cues, and imaginal exposure. The current findings suggest that increasing emotional arousal, in fact, increases activation in the distributed cognitive control network, particularly fronto-parietal regions. Taken together, these findings suggest that varying emotional arousal during rTMS may be a productive means of strengthening the response within the distributed cognitive control network.
Delivery of therapeutic doxorubicin dose across the canine blood-brain barrier with hyperthermia and temperature-sensitive liposomes

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Funding Sources: Hollings Cancer Center’s Cancer Center Support Grant P30 CA138313. NIH grant R01CA181664.

Category: Other

Background/Purpose:
Delivering chemotherapeutic drugs across the blood brain barrier (BBB) is a major challenge in the treatment of brain tumors. The BBB can be transiently opened by the application of hyperthermia (>40 °C). Thermosensitive liposomal doxorubicin (TSL-Dox) is a drug delivery system that rapidly releases the contained drug in response to hyperthermia. The goal of this study was to demonstrate delivery of doxorubicin across the BBB by TSL-Dox combined with local hyperthermia.

Methods:
TSL-Dox was infused intravenously over 30 min at a dose of 0.94mg/kg in anesthetized beagles (age ~17 months). Following, a hyperthermia probe was placed 5-10 mm deep through one of 4 skull burr holes. Hyperthermia was performed randomized for 15 or 30 minutes, at either 45 or 50 °C. Blood was drawn at baseline, immediately after completion of doxorubicin infusion, and then every 30 min for up to 180 minutes. Non-survival studies were performed in four dogs, where brain tissue at the hyperthermia location was extracted following treatment to quantify doxorubicin uptake via HPLC, and to visualize cellular uptake via microscopy. Survival studies for 6 weeks were performed in 5 dogs treated by a single hyperthermia application.

Results:
Local doxorubicin delivery ranged from 0.11 to 0.74 ng/mg of brain tissue at the hyperthermia locations, with undetectable drug uptake in unheated tissue. Fluorescence microscopy demonstrated cellular doxorubicin uptake. Histopathology in H&E stained samples demonstrated localized heat-induced damage near the probe. No animals in the survival group demonstrated significant neurological deficits.

Conclusion:
Localized doxorubicin delivery to the brain can be facilitated by TSL-Dox with localized hyperthermia with no significant neurotoxicity.
Elevated local production of complement components C3/C3a suppress sinonasal vitamin D3 metabolism in patients with chronic rhinosinusitis

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Category: KL2 Scholar

Background: Previously we demonstrated that patients with chronic rhinosinusitis with nasal polyps (CRSwNP) have alterations to the vitamin D system that extends beyond a nutrient deficiency. CRSwNP patients have reduced sinonasal levels of the active metabolite of VD3, 1,25(OH)2D3, even with adequate circulating forms of its precursors or 1,25(OH)2D3 itself. Furthermore, reductions in human sinonasal epithelial cell (HSNEC) levels of 1α-hydroxylase, the enzyme responsible for the final hydroxylation to make 1,25(OH)2D3, is associated with more severe CRSwNP. The mechanism suppressing local 25(OH)D3 to 1,25(OH)2D3 metabolism remains unknown and therefore is the focus of these studies.

Results: Conditioned media from CRSwNP, but not control HSNECs, suppressed HSNEC 1α-hydroxylase, suggesting a HSNEC-produced mediator was responsible for 1α-hydroxylase suppression. Given reports that CRSwNP HSNECs had elevated production of the complement components C3/C3a, we examined its roles in 25(OH)D3 metabolism. CRSwNP sinonasal tissue explants expression of 1α-hydroxylase was found to inversely correlate with C3, but with none of the other 11 previously described regulators of 25(OH)D3 metabolism. In vitro studies demonstrated that recombinant C3a, the cleavage product of C3, resulted in the suppression of HSNEC 1α-hydroxylase. Conversely, treatment of HSNEC with C3aRA, blocked antigen-induced suppression of 1α-hydroxylase and 25(OH)D3 to 1,25(OH)2D3 metabolism.

Conclusions: These studies are the first to demonstrate C3/C3a involvement in the regulation of 25(OH)D3 metabolism. These studies also provide mechanistic insights into how patients with CRSwNP have reduced local 1,25(OH)2D3, which may be responsible for the lack of efficacy of oral VD3 supplementation in improving clinical outcomes in patients with CRSwNP.

JKM and these studies are supported by the South Carolina Clinical & Translational Research (SCTR) Institute, with an academic home at the Medical University of South Carolina, NIH/NCATS Grant Numbers KL2 TR001452 & UL1 TR001450.
The Role of Lysyl Oxidase in Systemic Sclerosis-Associated Lung Fibrosis

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Category: TL1 Trainee

Introduction: Systemic sclerosis (SSc) is a connective tissue disease of unknown etiology characterized by progressive fibrosis of the skin and multiple visceral organs. Effective therapies for SSc are needed. Lysyl oxidase (LOX) is a copper-dependent amide oxidase that plays a critical role in the crosslinking of the extracellular matrix (ECM). In this study, we investigated the role of LOX in the pathophysiology of SSc.

Results: LOX mRNA was increased in lung tissues and matching fibroblasts of SSc patients. rLOX induced ECM production in vitro and ex vivo in lung fibroblasts and in human lung tissues maintained in organ culture, respectively. Additionally, TGF-beta and bleomycin induced ECM production, LOX mRNA expression and activity. Endostatin peptide abrogated these effects. In vivo, rLOX synergistically exacerbated pulmonary fibrosis in bleomycin-treated mice. The inhibition of LOX catalytic activity by BAPN failed to abrogate LOX-induced ECM production. LOX increased the production of IL-6. IL-6 neutralization blocked the effects of LOX. Further, LOX induced c-Fos expression and its nuclear localization.

Conclusions: LOX expression and activity were increased with fibrosis in vitro, ex vivo, and in vivo. LOX induced fibrosis via increasing ECM, IL-6 and c-Fos translocation to the nucleus. These effects were independent of the crosslinking activity of LOX and mediated by IL-6. Our findings suggest that inhibition of LOX may be a viable option for the treatment of lung fibrosis. Further, the use of human lung in organ culture establishes the relevance of our findings to human disease.

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Direct correlation between plasma microbial translocation and autoantibodies in first-degree relatives of patients with systemic lupus erythematosus

Elizabeth Ogunrinde, BS, Graduate Student, Microbiology & Immunology

Category: Other

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production against self-antigens. However, the process of events underlying autoantibody formation in SLE remain unclear. The objective of this study is to investigate the relationship between plasma autoantibody levels and plasma microbial translocation using first-degree relatives (FDRs) of SLE patients as a model in comparison to unrelated healthy controls (UHCs).

Method: Plasma samples from 36 female African-Americans (18 UHCs and 18 FDRs) were assessed for autoantibody levels by autoantibody array, lipopolysaccharide (LPS) levels by the limulus amebocyte assay and microbiome composition by 16S rDNA analysis.

Results and Conclusion: Compared to UHCs, FDRs exhibited elevated levels of plasma autoantibodies, and parents and children of lupus patients exhibited elevated levels of plasma LPS. Plasma LPS levels were positively correlated with plasma autoantibody levels in FDRs but not in UHCs. Circulating microbiome analysis using plasma samples revealed reduced microbiome diversity in FDRs compared to UHCs, and UHCs exhibited a higher relative abundance of Streptococcaceae compared to FDRs after adjusting for multiple comparisons. Taken together, these results indicate a possible role for plasma microbial translocation and microbiome composition in influencing autoantibody development in SLE.
A Family-Centered Self-Management Program for Young Children with Sickle Cell Disease

Shannon Phillips, PhD, RN, Assistant Professor, Nursing

Category: KL2 Scholar

**Background:** Sickle cell disease (SCD) affects ~100,000 individuals in the US and can result in multiple acute and chronic negative health outcomes including: symptoms such as pain and fatigue, organ damage, and increased hospital admissions and ED visits. Children with SCD and their families could benefit from self-management strategies that reduce negative outcomes.

**Results:** Key informant interviews were conducted with 10 HCP and 12 parent/child dyads to obtain feedback on an mHealth intervention designed to improve self-management skills among children with SCD ages 0 - 7. Data were collected using qualitative description, and were analyzed using a deductive-inductive approach. Participants reviewed each of the three components of the intervention - electronic educational materials, symptom monitoring and tracking, and patient-provider communication – and provided suggestions for improvement and insights on the usefulness of the intervention. All participants described self-management behaviors that would be improved by the intervention, and most of the dyads reported they would use the intervention every day or nearly every day.

**Summary:** While participants suggested improvements to the intervention, they also perceived it would be useful and acceptable for children with SCD and their families. Conclusions: Findings informed revisions to the intervention, and will inform phase II of the study, in which feasibility testing of the intervention will be conducted with 30 parent/child dyads. This intervention has potential for improving self-management and reducing symptoms such as pain and fatigue in a broad population of children with SCD.
Comprehensive Identification of Differentially Methylated Regions Associated with Systemic Sclerosis in Dermal Fibroblasts from African-American Patients

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Category: Other

Background. The etiology and reasons underlying the ethnic disparities in systemic sclerosis (SSc) remain unknown. African-Americans (AA) are disproportionally affected by SSc, yet dramatically underrepresented in research. The role of DNA methylation in disease risk remains unclear. This analysis was conducted to comprehensively identify differentially methylated loci associated with SSc in AA.

Methods. Genomic DNA was isolated from cultured dermal fibroblasts isolated from 15 AA SSc cases and 15 AA controls. All patients met the 2013 ACR/EULAR classification criteria for SSc. DNA methylation patterns were profiled through reduced representation bisulfite sequencing (RRBS). Alignment and methylation calling were performed using Bismarck v0.16.3 and the GRCh37/hg19 reference genome. Data was filtered, normalized, and analyzed with RnBeads v1.6.1.

Results. We generated DNA methylation data for over 3.8 million CpGs. Using RnBeads’ Combined Score approach, a total of 97 CpG islands, 197 genes and 112 promoters showed significant differential methylation levels between cases and controls. The top differentially methylated genes constitute mostly non-coding RNA genes (42%), followed by pseudogenes (27%), then protein coding genes (19%). Enrichment analysis revealed that both hypo- and hypermethylated genes and their promoter regions were enriched for cell differentiation and immune-related gene ontology terms.

Conclusions. We observed modest DNA methylation differences between cases and controls, mostly in non-coding RNA genes, supporting a larger contribution of dysregulated regulatory elements to disease. While previous DNA methylation profiling analyses in European-Americans reported an enrichment of extracellular matrix and focal adhesion genes, our data supports a potentially stronger immune-driven etiology in AA.
CD45+/ COL I+ Cells In Lung Fibrosis

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Category: Other

Background: It is controversial whether the role of cells of the hematopoietic lineage in fibrosis is solely due to monocytes differentiating into macrophages that activate resident fibroblasts, or whether monocytes also differentiate into fibrocytes (CD45+/ COL I+ cells) that then differentiate into fibroblasts. We previously showed that in the fibrotic lung, fibrocytes are present in greater numbers than in control tissue. We also showed that both fibroblasts and monocytes from humans and mice with fibrotic disease are deficient in caveolin-1, leading to enhanced monocyte recruitment and differentiation into fibrocytes and enhanced COL I production by fibroblasts. The effects of low caveolin-1 are reversed in vitro and vivo by the caveolin-1 scaffolding domain peptide (CSD).

Results: We have extended these studies using flow cytometry and transgenic mice to further relate the hematopoietic lineage and fibrocytes/ fibroblasts. We find the levels of several macrophage markers are higher on CD45+/COL I+ fibroblasts from fibrotic lung than from control lung. To demonstrate that CD45+/COL I+ fibroblasts actually express COL I, we analyzed mice expressing EGFP under the control of the COL Iα1 promoter. Indeed, CD45+ fibroblasts were EGFP+ indicating that they express COL I. The planned use of Cre technology will provide more convincing evidence that CD45+ fibroblasts are of hematopoietic origin and enable the determination of whether the therapeutic effects of CSD on lung fibrosis are on fibrocyte accumulation, their differentiation into fibroblasts, and/or the production of COL I by fibroblasts.

Conclusions: Evidence is building that a major portion of the myofibroblasts in fibrotic tissue are of hematopoietic origin.
Glycosphingolipids as Biomarkers of Lupus Nephritis

Jessalyn Rodgers, MS, Research Specialist II, Medicine, Rheumatology

Category: Other

Background: Glycosphingolipid (GSL) levels and activity/expression of neuraminidase (NEU), which mediates GSL catabolism, are elevated in kidneys and/or urine of lupus mice and nephritic human patients compared to controls. Exosomes, 20-100nm extracellular vesicles, contain lipids, proteins and RNA representative of the cells from which they were derived and are abundant in human urine. Thus, exosomes are a potential source of biomarkers of renal disease in lupus nephritis (LN) patients. We hypothesize: 1) levels of GSL molecules may be potential biomarkers of flare and therapeutic response and 2) decreasing NEU activity will reduce proteinuria and/or progression of nephritis in lupus.

Results Summary: Preliminary results measuring GSL levels in exosomes from LN patient urine showed significant differences between patients who responded or failed to respond to treatment. A pilot study of urine exosomes from five LN patients taken during quiescent disease and a disease flare and from five lupus non-nephritic patients show: 1) increased GSL levels in flare samples compared to non-flare, and/or control samples; and 2) differences in the levels of proteins between flare, non-flare, and/or control samples. We have generated a NEU1 heterozygote (Neu1+/-) on the B6.SLE1/2/3 lupus prone mouse strain, and are assessing for effects on disease development. Preliminary data indicate that the Neu1+/- lupus mice have decreased/delayed development of proteinuria.

Conclusions: Data suggests that molecules in this pathway may serve as biomarkers of flare and/or response to therapy in LN patients and GSL catabolism as a potential target for therapeutic intervention.
Identifying the Role and Immunobiological Mechanisms of Fli-1 Mediated Pathogenicity in Graft-versus-Host Disease

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Category: TL1 Trainee

Background: Allogeneic hematopoietic stem cell transplantation (allo-HCT) is a curative procedure for hematological malignancies. Chronic graft-versus-host disease (cGVHD) is a lethal complication that often develops after allo–HCT. Fli-1 is an aberrantly expressed protein in cancers including erythroleukemia and melanoma, while being implicated in pathogenesis of systemic lupus in mice and humans, a disease with marked similarity to cGVHD. cGVHD was induced using hematopoietic cells from conditional knock-out mice deficient for the fli-1 gene specifically on T cells and progression of cGVH in murine allo-HCT recipients was monitored using a clinical scoring system, and changes in activation status of hematopoietic cell populations were quantified using flow cytometry.

Results Summary and Conclusions: Recipients transplanted with fli-1 deficient T cells exhibited significantly reduced cGVHD clinical scores compared to littermate wild-type controls. Donor-grafts containing fli-1 deficient T cells were also associated with restrained T-cell responses including reduced Interferon-γ cytokine production, PD-1 expression, and differentiation into follicular helper T cells. fli-1 T-cell deficient donor-grafts also improved donor B-cell reconstitution and reduced plasma cells in allo-HCT recipients relative to littermate wild-type control donor-graft recipients. Thus, inhibiting Fli-1 represents a promising therapeutic strategy for the goal of preventing cGVHD after allo-HCT while also directly targeting cancers which aberrantly express Fli-1.
Local Causal Networks Discover Predictive Cytokine Biomarkers of Scleroderma

Ali Shojaee Bakhtiari, PhD, Research Associate, Medicine, Public Health Sciences
Trainee: Other

Background

Scleroderma is an autoimmune disease with established relationship to immune cytokines. The purpose of this work is to discover cytokine biomarkers of scleroderma using local causal neighborhood (LCN) learning techniques.

Materials, Methods and Results

40 cases of positive scleroderma patients and 24 healthy controls. We measured 29 bronchoalveolar lavage fluid (BAL) cytokines. We preprocessed the data and properly imputed the missing values and adjusted for sex and race. We performed univariate Welch t-tests to find the cytokines significantly associated with scleroderma status (SS). We used the HITON-PC algorithm from causal explorer toolbox to identify the local causal network (LCN) of SS. We used multiple runs of cross-validation to assess the stability of the biomarker identification. We also performed predictive analysis using logistic regression model of cytokine biomarkers on SS. The performance was assessed using the AUC of the ROC curve. We found 8 cytokines significantly associated with SS (P<0.05). HITON-PC algorithm identified 2 cytokines to be in the LCN of the SS. Cross validation of the HITON-PC showed that the selected cytokines are consistently present in the LCN. The predictive performance of the LCN biomarker achieved an average classification success rate of 68.46%, mean sensitivity of 65.49% and mean specificity 73.44%. AUC of ROC curve is 73%.

Conclusions

In this work, we demonstrated the use of LCN for discovering cytokines biomarkers of scleroderma status. We showed the merit of using the LCN, by rigorously testing the model's predictive power.
A novel cell-based assay for diagnosing recurrent FSGS

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Category: Other

Background: FSGS (focal and segmental glomerulosclerosis), is a disease that primarily targets kidney podocytes (an important constituents of kidney’s filtration barrier) and whose dysfunction leads to progressive renal failure. Here, we report the development of a human podocyte cell-based assay that will serve as a non-invasive diagnostic clinical tool to detect recurrent FSGS. The concepts and approaches demonstrated are widely applicable in designing assays for other forms of FSGS, which are the leading causes of ESRD and their diagnostic gold standard remains the invasive kidney biopsy method. This assay is specifically aimed at diagnosing rFSGS to avert the ineffective renal transplant in FSGS patients.

Results Summary: We identified rFSGS responsive genes by profiling (RNASeq) human podocytes treated with plasma derived from human rFSGS and control patients, which also induced significant alterations to podocyte actin cytoskeleton partially mimicking the disease processes. Two unique candidate genes (proprietary information) based on profiling data from control and rFSGS patients were selected. Next, their promoter regions were cloned into a promoterless reporter vector, transduced into podocytes and luciferase assay was performed. This assay allowed us to measure plasma-induced increase in luminescence in these cells.

Remarkably, both cell lines showed similar results, where only rFSGS patient plasma showed ~2-fold induction, whereas no induction was observed with plasma from other nephropathies including minimal change disease (MCD), membranous glomerulonephritis (MGN) and FSGS (Fig1).

Conclusions: The developed assay is noninvasive, sensitive, specific, accurate and studies are being planned for conducting clinical trials to utilize its full diagnostic potential.
PDGFR-ß+ Cells: a Novel Reservoirs for HIV in the Lungs

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Category: Other

Background: The success of anti-retroviral therapy (ART) in HIV patients has lengthened life span. However, HIV infection is not completely eradicated due to reservoirs of virus. The lung has been proposed as an important reservoir of HIV. This may contribute to the increased risk of chronic pulmonary complications, including COPD. The lung pericyte is a mesenchymal-derived cell that is critical for vascular homeostasis, through secretion of pro-survival molecules such as angiopoietin-1 (Angpt-1). It has been shown in vitro that HIV can directly infect brain pericytes, leading to reduced endothelial cell association and increased leakiness of the blood-brain barrier. We hypothesize that lung pericytes can also be infected by HIV and may serve as a reservoir for the virus. Furthermore, we propose that HIV infection switches pericytes to an activated state in which Angpt-1 secretion is decreased and pro-inflammatory cytokine production is increased. To test this hypothesis, we isolated pericytes from digested human lung tissue by selecting for PDGFR-ß, a common marker for pericytes.

Results: We found that lung pericytes express key co-receptors for HIV, including CD4, CXCR4 and CCR5. Furthermore, lung pericytes are directly infected by HIV-1, as determined by p24 production and RT-PCR for HIV-1. Infected pericytes show increased expression of the pro-inflammatory genes CXCL1, CXCL2, CXCL10 and IL-8. In addition, HIV infection decreased Angpt-1 expression and, following exposure to TGFß1, transiently decreased myofibroblast markers.

Conclusion: Taken together, our data demonstrate that HIV directly infects lung pericytes, which may represent a previously unrecognized reservoir of HIV in the lung.
Maternal Cardiometabolic Determinants of Breastfeeding Noninitiation in South Carolina by Maternal Race and Ethnicity

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Category: TL1 Trainee

Background: In order to inform targeted clinical interventions, we sought to identify maternal cardiometabolic determinants of breastfeeding noninitiation by race/ethnicity.

Results Summary: Our study population is comprised of 120,771 non-Hispanic whites (NHW), 64,877 non-Hispanic Blacks (NHB), and 20,084 Hispanics with live singleton births in South Carolina delivered at a gestational age between 37-44 weeks from January 2004 to 2008. Logistic regression was used to evaluate the association between maternal cardiometabolic factors and breastfeeding noninitiation by hospital discharge, with stratification by race/ethnicity to examine race/ethnic differences in this population. Compared to NHW and Hispanics, NHB were more likely to be overweight or obese, hypertensive, and/or diabetic entering the pregnancy. Breastfeeding noninitiation was also higher among NHB (NHW: 31.5%, NHB: 56.8%, Hispanics: 14.6%). In full models, all race/ethnicities were significantly less likely to initiate breastfeeding if they were obese or diabetic prior to pregnancy, or gained inadequate weight during the pregnancy. Hypertension was significantly associated with higher rates of breastfeeding noninitiation among NHW (OR: 1.06, 95% CI: 1.01, 1.17) and NHB (OR: 1.07, 95% CI: 1.02, 1.13), but not Hispanics. NHB women were significantly less likely to initiate breastfeeding if impacted by gestational diabetes (OR: 0.92, 95% CI: 0.86, 0.98) or excessive weight gain during pregnancy (OR: 0.93, 95% CI: 0.90, 0.97).

Conclusions: Our study shows that breastfeeding noninitiation in South Carolina varies by maternal cardiometabolic factors and race/ethnicity. This study can aid in the development of tailored clinical and public health breastfeeding interventions and improve maternal and child health.
Glycosphingolipid Catabolism Mediates Mesangial Cell IL-6 production

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Category: Other

Background:

Glycosphingolipid (GSL) levels and neuraminidase (NEU) activity/expressions are elevated in the kidneys and/or urine of lupus mice and human patients with proliferative nephritis compared to their non-nephritic counterparts and healthy controls. Elevated renal GSL catabolism suggests this pathway may play a role in mediating the pathogenesis of lupus nephritis.

Results and Summary:

Using primary MCs from lupus prone MRL/lpr mice, we demonstrated significant and dose-dependent increases in IL-6 production following stimulation with the immune complex mimic heat aggregated IgG (HA-IgG) and lupus serum which is blocked by the NEU inhibitor oseltamivir phosphate (OP). Preliminary data show that NEU activity and IL-6 production were not significantly increased in MCs derived from C57BL/6 Neu1+/− mice compared to MCs derived from C57BL/6 Neu1+/+ MCs. Further evidence that NEU activity mediates IL-6 production by MCs was observed in the immortalized MES 13 MC line. Overexpressing NEU1 or NEU3 in MES 13 MCs resulted in a significant dose-dependent increase in IL-6 production in the absence of stimulation. This IL-6 response was blocked by MAPK inhibitors suggesting NEU mediates IL-6 production through MAPK signaling. Co-localization studies detected overlapping expression of NEU1 and NEU3 with HA-IgG on the plasma membrane of primary MCs and with IgG deposits in renal sections of lupus mice.

Conclusion:

Together, these results suggest that increased GSL catabolism mediates MC production of IL-6 possibly by interacting with an IgG-receptor complex that triggers MAPK signaling. Targeting the GSL catabolic pathway may reduce renal cytokine production and inflammation in lupus nephritis.
Characterization of Metabolic Fitness of T cells in Tumors

Authors

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Category: Other

Abstract

Tumors inhibit effector function of T cells through multiple mechanisms, but the specific effect of tumor microenvironments on T cell metabolic activity has not been assessed. Assays that directly measure the level of T cell metabolic fitness in patient tumors have not been developed. We hypothesized that reactive oxygen species produced within the mitochondria (mtROS) as a byproduct of oxygen consumption within the electron transport chain may be an indicator of metabolic fitness of T cells in tumors. We used the fluorescent dye mitosox red to measure accumulation of superoxide anions within mitochondrial membranes of T cells. We found that antigen-specific activation of mouse or human T cells led to accumulation of mtROS in CD8\textsuperscript{+} T cells. Glutathione, a key cell-intrinsic anti-oxidant necessary for T cell inflammatory function, was preferentially consumed by mtROS\textsuperscript{+} T cells. Treatment of T cells with extrinsic anti-oxidant N-acetylcysteine reduced mtROS production in T cells, extinguished glutathione uptake, and reduced inflammatory function. Assessment of mtROS in CD8\textsuperscript{+} TILs from multiple patient tumor types and mouse models showed a consistent lack of mitochondrial function of T cells in tumors. Importantly, PD-1\textsuperscript{+} CD8 TILs expressed low levels of mtROS. We found that tumor microenvironment conditions of hypoxia and nutrient deprivation extinguished mtROS expression and this led to loss of inflammatory cytokine production. Inhibition of tumor nutrient supply through angiogenic inhibition led to a 4-fold increase in mtROS in CD8\textsuperscript{+} TILs. Increased metabolic fitness of effector T cells promoted response to anti-PD-1 therapy in a mouse melanoma model. Therapies that remodel tumor microenvironments to restore T cell metabolic function may improve the efficacy of anti-PD-1 treatment and expand the range of patients able to respond to such therapy.
Discovery and Evaluation of FOXP3 Dimerization Inhibitors

Ravyn M. Thompson, Graduate Student, Cell & Molecular Pharmacology, Cara Coleman, Nathan G. Dolloff

Category: TL1 Trainee

Immuno-oncology (IO) strategies are promising new approaches for the treatment of a variety of malignancies, including multiple myeloma (MM). Regulatory T cells (Tregs), which suppress effector T cell function, are a limitation to durable IO responses. The transcription factor FOXP3 is critical for the mature Treg phenotype. FOXP3 homodimerization is required for DNA binding and transcriptional activity, and mutations mapping to the dimerization region are associated with IPEX syndrome, resulting in dysfunctional Tregs in humans. We therefore hypothesize that inhibitors of FOXP3 dimerization will repress Treg suppression and enhance the anti-MM activity of IO. To discover FOXP3 dimerization inhibitors, we are modeling FOXP3 homodimerization in vitro. Currently, we are optimizing an ALPHA screen and an ELISA-based dimerization assay using recombinant full length and truncated versions of FOXP3 to discover peptidomimetics that inhibit homodimerization. Induced Tregs expanded from human PBMCs will be treated with lead biologics and functional assays will be performed. Here we demonstrate Treg suppression of T cell proliferation and IFN-γ secretion after 5 days of coculture under basal conditions. Additionally, we developed a MM/T cell co-culture system to measure anti-MM T cell responses and show decreased anti-MM T cell activity in the presence of Tregs. We expect to exploit the assays outlined here to demonstrate defective Treg suppression when FOXP3 dimerization is inhibited. These studies support drug discovery efforts that will ultimately improve IO therapies for patients with MM.
Combination Therapy of SSc Using MSCs and the Caveolin-1 Scaffolding Domain Peptide Background

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Category: Other

The potential value of MSC therapy in treating skin fibrosis in scleroderma (systemic sclerosis, SSc) and of the caveolin-1 scaffolding domain peptide (CSD) in treating lung, skin, and heart fibrosis in mouse model systems has been observed. However, there has been little work reported on treating lung fibrosis with MSCs or in combining MSCs with other therapies. We have characterized the altered fibrogenic and adipogenic potential of adipose-derived MSCs (AT MSCs) from humans and mice with fibrotic disease. We further determined the beneficial effect in mice of combined AT MSC and CSD therapy. Results. SSc AT MSCs have a profibrotic/anti-adipogenic phenotype compared to healthy AT MSCs (low caveolin-1, high ASMA, low ability to be induced to differentiate into adipocytes). This phenotype is mimicked by treating healthy AT MSCs with TGFβ or caveolin-1 siRNA and is reversed by CSD. Similar results were obtained with SSc AT MSCs and AT MSCs from bleomycin-treated mice. When bleomycin-treated mice received MSC injections, both fibrotic and healthy MSCs had a beneficial effect on skin fibrosis that was not further enhanced by CSD. However, CSD did synergize with both fibrotic and healthy MSCs in having a beneficial effect on lung fibrosis. Immunohistochemical studies on fibrosis markers in the lung confirmed the synergistic beneficial effect of combined CSD and MSC treatment. Conclusions. CSD has a synergistic beneficial effect with MSC treatment due to its inhibition of MSC differentiation into myofibroblasts. This effect may also involve the ability of CSD to alter the environment in vivo (ECM, cytokines), thereby affecting MSC fate.
Relationship Power Imbalance and History of Male Partner HIV Testing Among Pregnant Women in Central Uganda

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Category: TL1 Trainee

Background: In certain societies, greater power is given to males over females in heterosexual relationships, and this imbalance can have a negative impact on health, including HIV prevention. We investigated the association between relationship power imbalance and male partner HIV testing, using baseline data from a HIV self-testing trial in three antenatal clinics in central Uganda. Pregnant women with HIV- male partners were recruited and randomized by day into standard of care or intervention (HIV self-testing kits). Analyses were performed in SAS 9.4, with χ² tests and p<0.05 for significance.

Results: 1,514 women were recruited (737 standard of care, 777 intervention). Overall, 39.6% of male partners had previously tested for HIV. Among women <26, contributions to expenses differed by partner testing (overall p<0.001, 47.6% of women whose partners tested made no contribution vs. 63.2% of women whose partners did not test). Relationship status differed by partner testing (overall p=0.02, 12.4% of women whose partners tested showed a sometimes difficult relationship vs. 5.7% of women whose partners did not test). Among women 26+, decision making for family visits differed by partner testing (overall p=0.005, 52.9% of women made joint decisions with partners who tested vs. 36.5% whose partners did not test).

Summary and Conclusions: Higher relationship power balance was associated with higher HIV testing among male partners when measured by contribution to expenses and decision making for family visits, but not relationship status. Relationship power balance should be considered when counseling women and men to increase HIV testing.
Harvesting Human Islets in Carbon Monoxide-saturated Medium Enhances Insulin Independence after Islet Autotransplantation

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Category: SCTR Pilot Project

Stresses encountered during human islet isolation and post-transplantation leads to islet cell death after transplantation, which reduces the chance of insulin independence in chronic pancreatitis (CP) patients undergoing total pancreatectomy and islet autotransplantation (TPIAT). We tested whether harvesting islets in carbon monoxide (CO)-saturated solutions can enhance islet survival and insulin independence after TPIAT in participants without pre-existing diabetes. CP patients consented to the study were randomized into CO (islets harvesting in CO-saturated medium) or control (islet harvesting in normal medium) groups. Islet yield and oxygen consumption rate (OCR) were measured before transplantation. Diabetes onset and insulin requirement were measured at 6 months post-transplantation and used as the primary efficacy outcome. At month 6, 37.5% (3 in 8) CO and none (0 of 5) patients were insulin independent. CO-islets showed significantly higher OCR value before transplantation. Patients receiving CO islets had reduced serum CXCL23 and increased CXCL12 levels at 1 and 3 days post transplantation compared to controls, suggesting CO exposure increased islet viability/quality and caused less inflammation after transplantation. Our current finding show for the first time that harvesting human islets in CO-saturated solutions increased insulin independence in CP patients undergoing TP-IAT, and provide a potential mechanism of CO protection. Clinicaltrials.gov registration number: NCT02567240.