

Sixth Annual Translational Research Symposium
In Memory of David Seldin, MD, PhD
March 20, 2017

Poster Presentations

This year's poster session incorporated 40 ePoster board presentations illustrating a broad range of translational science topics. All trainee posters were judged on eight different criteria, resulting in five poster winners, including one receiving a top score and a \$1,000 prize. The five winners are listed below, followed by abstracts of the posters presented at the symposium. Congratulations to the winners and to all the participants.

POSTER SESSION WINNERS

➤ **1st Place:**

(18) Richard Giadone, "Genome Editing of iPSCs for Treating Familial Transthyretin Amyloidosis"

➤ **2nd Place:**

(5) Elizabeth Becker, "Lung function decline"

➤ **3rd Place (3 Way Tie):**

(3) Konstantinos-Dionysios Alysandratos, "Generating AT2 cells from PSC to model surfactant protein C mutations"

(23) Killian Hurley, "Modelling AATD Lung Disease Using Alveolar Epithelial Cells Derived from iPSC"

(36) Yuliang Sun, "Using patient-derived induced pluripotent stem cells to model ABCA3 dysfunction"



1. BU-i2B2 4 U? INTRODUCTION TO A LARGE CLINICAL DATA RESOURCE FOR BOSTON

WILLIAM G. ADAMS, MD, SARA CURRAN, AND CHRISTOPHER SHANAHAN, MD

Overview: There is a pressing need to provide access to clinical data for research in a way that is easy to use and secure. The clinical and translational research community has laid the ground work for such a system via its broad adoption of “Integrating Informatics from Biology to the Bedside (i2b2)”. Over the past 8 years, researchers at the BU Medical Campus have successfully created and now maintain an i2b2 instance for Boston Medical Center (BMC) and 8 affiliated Community Health Centers (CHC). Data in i2b2 is de-identified to preserve privacy but dates are available for each data element. Users of i2b2 can do rapid, self-service queries as preparatory to research. They can also request data extracts. More recently, the BU-CTSI team has extended our instance of i2b2 by developing a new i2b2 Cell, the Health Outcome Monitoring and Evaluation (HOME) Cell which supports much more complex logic and data generation for health outcomes over time.

In this presentation we seek to provide a high-level view of BU-i2b2 by describe the data sources used in the system, software available for queries and data exploration, and review of sample findings from several projects.

Discipline: Clinical Research Informatics, Health Outcome and Clinical Effectiveness Research

Objectives/Goals: The primary objectives for our team focus on supporting the work of clinical researchers who seek to explore a wide variety of questions that require large amounts of data in a standardized form but do not require identification of patients. The tools and data we provide support health services research, comparative effectiveness research, disparities research, and improvement/implementation science activities.

Methods/Study Population: i2b2 is an open-source data architecture based on the Massachusetts General Hospital Research Patient Data Repository. Data is extracted from multiple sources (Electronic Health Records (EHR) and registration/billing systems primarily) and transformed into a standards-based data structure using an master patient index (all records across sites are linked for an individual). Via the i2b2 Query Cell and HOME Cell web-clients users are able to explore the data via a “drag-and-drop” interface. Our data covers a very large portion of the population of South End, South Boston, Dorchester, Roxbury, Mattapan, and Roslindale.

Results/Anticipated Results: Over the past 8 years we have supported a variety of research projects including: assessment of predictors of visit-to-visit variation in blood pressure control, multi-site assessment of comparative effective of medication used for common chronic health conditions, predictive analytics algorithms for predicting cardiac events, assessment of community violence and childhood asthma, declines in HPV morbidity associated with vaccination, and many other projects. Going forward we seek to collaborate with many more researchers, particularly those in informatics, engineering, and health services on the Charles River Campus as well as the School of Public Health

Discussion/Significance of Impact: Our activities related to BU-i2b2 address multiple national priorities related to data sharing, clinical research informatics, health disparities, and health services research. Our new web-client version of the HOME Cell substantially improves our community’s access to HOME Cell functionality and is a novel, sharable resource for use within the CTSA/NCATS community. The HOME Cell provides a new way to perform large-scale collaborative research without the need to actually move patient-level data.



Our efforts support multiple strategic goals including: 1) support for building national clinical and translational research capacity by enhancing a broadly adopted informatics tool (i2b2); 2) enhanced consortium-wide collaborations by offering a tool that can be easily shared within the CTSA Network to support multi-institutional collaboration; and 3) improving the health of our communities by offering a tool that has the potential to provide new insights into health care processes and outcomes that could drive innovation and improvement activities.

We encourage researchers within the BU community with interest in using our services to visit <http://sites.bu.edu/bu-i2b2/> or contact badams@bu.edu.

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2. B-CATENIN/CBP AXIS DRIVES CANCER INITIATING CELLS IN OSCC

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Objectives: Oral squamous cell carcinoma (OSCC) is an aggressive malignancy characterized by molecular heterogeneity and locoregional spread associated with high morbidity. Aggressive cancers are thought to arise from populations of cancer initiating cells (CICs) that exhibit the properties of stem cells and drive tumor development, recurrence and resistance to therapy. The transcriptional regulator, β -catenin, has been implicated in OSCC CICs. Nuclear β -catenin has been shown to recruit the chromatin remodeling CREB binding protein (CBP) to drive expression of proliferation and survival genes, as well as genes that maintain stem-like phenotypes. We hypothesized that targeting β -catenin-CBP interaction will inhibit CICs in oral tumors and restore an epithelial phenotype.

Methods: To test tumor aggressive potential of OSCC CICs, we used zebrafish as a model system. We isolated CD44⁺CD24^{hi}CD29⁺ cells from aggressive HSC-3 OSCC cells by FACS and assayed their ability to drive tumor growth and metastases in zebrafish compared to unsorted and CD44⁺CD24^{low}CD29⁺ cells. In addition, we examined the role of the β -catenin/CBP axis in the aggressive phenotype of these cells. We also assessed whether the β -catenin/CBP axis affected CICs in tumors from immune competent HPV⁺ mice.

Results: Zebrafish injected with subpopulation of cells co-expressing CD44⁺CD24^{hi}CD29⁺ primitive cell surface markers drove rapid tumor growth and metastases, followed by unsorted and sorted CD44⁺CD24^{low}CD29⁺. Treatment of CD44⁺CD24^{hi}CD29⁺ cells with a small molecule inhibitor of the β -catenin-CBP interaction, ICG-001, interfered with tumor growth and metastases in zebrafish. Further, ICG001 inhibited tumor growth in immunocompetent HPV⁺ murine model. On a cellular level, ICG-001 promoted membrane localization of β -catenin, enhanced E-cadherin adhesion and restored epithelial phenotype.

Conclusion: Our studies indicate that the β -catenin/CBP axis promotes OSCC CICs and that ICG-001 may be an effective therapeutic agent for this malignancy.



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3. GENERATING ALVEOLAR TYPE 2 CELLS FROM PLURIPOTENT STEM CELLS TO MODEL SURFACTANT PROTEIN C (SP-C) MUTATIONS *IN VITRO*

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Introduction: Childhood interstitial lung disease (chILD) is a group of parenchymal lung diseases presenting in infants, children, and adolescents. A subset of chILD patients has autosomal dominant mutations in the surfactant protein C (SFTPC) gene, expressed exclusively by alveolar type 2 (AT2) cells. SFTPC mutations are categorized into BRICHOS and non-BRICHOS. Overexpression of the most common non-BRICHOS SFTPC mutation I73T (SFTPC^{I73T}) in *in vitro* models is associated with surfactant protein mistrafficking and impaired metabolism. Because access to AT2 cells from chILD patients is limited and these cells transdifferentiate in culture, we sought to engineer an *in vitro* mouse embryonic stem cell (mESC) model for the production of putative AT2 cells to study the SFTPC^{I73T} mutation.

Methods: Using a directed differentiation protocol we generated Nkx2-1+ lung progenitors from mESCs that carry a targeted Nkx2-1^{mCherry} knockin reporter. To identify putative AT2 cells we transduced sorted Nkx2-1+ progenitors with a lentiviral vector carrying GFP under the control of a 3.7kb SFTPC promoter. Microarray RNA expression analysis and RT-qPCR were used to characterize the population of lung progenitor and putative AT2 cells.

Results: Sorting to purity and replating Nkx2-1+ lung progenitors after 14 days of *in vitro* differentiation led to the production of SFTPC^{GFP+} cells by day 25. Global transcriptomic profiling of day 14 Nkx2-1+ cells indicated that evolutionary conserved developmental genes involved in lung lineage specification are expressed. Purified day 25 SFTPC^{GFP+} cells expressed AT2-specific genes at levels comparable to primary fetal lung. We are now adapting this protocol to a novel mESC line containing the SFTPC^{I73T} mutation knocked-in to the endogenous SFTPC locus.

Conclusions: We show that generation of putative AT2 cells to potentially model chILD caused by SFTPC gene mutations is feasible. Employing this protocol, we will generate AT2 cells from SFTPC^{I73T} mutant mESC and compare them to wild type mESC to develop the first signature of chILD disease and potentially test novel therapeutics.

4. PARETIC PLANTARFLEXOR MUSCLE FUNCTION AFTER STROKE: IMPAIRMENTS IN ACTIVATION, NOT CAPACITY, CONTRIBUTE TO DEFICITS IN PARETIC PROPULSION



DURING HEMIPARETIC WALKING

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Introduction:

Although gait retraining is a major focus of rehabilitation after stroke, walking remains impaired after discharge due to a dearth of interventions that effectively target deficits in key walking subtasks. An inability to generate propulsion by the paretic limb is a major contributor to walking-related disability; however, few interventions have been able to target deficits in propulsion in a manner that improves walking function. This is, in part, due to heterogeneity in impairments and limitations in diagnosing the primary source of propulsion deficits. Indeed, although the ankle plantarflexor (PF) muscles are the primary generators of propulsion during walking and their function is impaired after stroke, it is unclear whether it is the reduced force-generating capacity of the PFs or reduced volitional access to the available capacity that primarily contributes to reduced propulsion during walking. The objective of this study was to investigate the relationship between paretic propulsion deficits and PF function after stroke. We hypothesized that impaired PF activation, not reduced capacity, underlies paretic propulsion deficits during walking.

Methods:

For 36 individuals > 6mo poststroke, PF capacity (i.e., maximum force generating ability (MFGA)), volitional strength (i.e., maximum volitional isometric contraction (MVIC)), and activation (defined as $MMMMMMMM \div MMMMMMMMM$) were measured using dynamometry and supramaximal electrical stimulation. Walking was analyzed on an instrumented treadmill with paretic propulsion defined as the peak anterior ground reaction force observed. Moderated regression quantified the relationships between PF function and paretic propulsion during walking, accounting for variation in speed across participants.

Results:

Deficits in paretic PF activation independently contributed to deficits in paretic propulsion. A walking speed x PF activation interaction was observed with the final model accounting for 81.7% of the variance in paretic propulsion ($R^2 = 0.817$, $F(3,32) = 47.56$, $P = 0.000$).

Conclusions:

Impaired activation of the full force-generating capacity of the paretic plantarflexors is predictive of paretic propulsion deficits during hemiparetic walking. Interventions that improve activation of the plantarflexors may be better positioned than traditional strengthening approaches to improve walking after stroke.

5. A BRONCHIAL AIRWAY GENE EXPRESSION SIGNATURE OF LUNG FUNCTION DECLINE

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the US and is characterized by varying rates of lung function decline and emphysema. Because the pathophysiologic determinants of lung function decline have not been identified, an individual's rate of decline of cannot be predicted. Previous work has identified a COPD-associated "field of injury" gene expression signature in bronchial airway epithelium. We hypothesized that this might reflect molecular alterations associated with the rate of lung function decline.



Methods: We generated a gene-expression dataset from bronchial airway epithelial brushings obtained within 1 year of bronchoscopy from ever smokers without cancer with ≥ 2 spirometry measurements performed ≥ 4 years apart ($n=134$). We identified genes whose expression in bronchial airway is associated with subsequent decline in FEV1 using a linear model. The relationship between the lung function decline signature and regional emphysema severity was examined using GSEA. The lung function decline signature was examined in an independent dataset of endobronchial biopsies from participants with COPD.

Results: Expression of 171 genes at baseline were significantly associated with subsequent lung function decline ($FDR < 0.05$). Genes whose expression increases with greater lung function decline also increase in lung regions with greater emphysema ($GSEA-FDR < 0.05$). Our gene expression signature is also concordantly associated with future lung function decline in an independent dataset ($p < 0.05$).

Conclusions: We have identified an airway gene expression signature associated with lung function decline. It is concordant with gene expression alterations associated with regional emphysema severity and an independent dataset of COPD patients, indicating the signature is consistent with a severe COPD phenotype across datasets. These findings suggest a potential utility for using airway epithelial gene expression for stratifying patients at risk for lung function decline in clinical trials of anti-COPD therapies and potentially for developing biomarkers to predict which patients will develop COPD.

6. THE ACUTE IMPACTS OF SMOKING ON AIRWAY GENE EXPRESSION

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Background: We have previously reported gene expression changes in the bronchial airway epithelium of active smokers with long-term exposure to tobacco smoke. In this study, we sought to describe the acute effects of smoking on airway epithelial gene expression.

Methods: Airway epithelial cells were collected from the airway via bronchoscopy from 63 individuals at baseline and 24 hours after acute smoke exposure (ASE) defined as 3 cigarettes in 1 hour. Gene expression differences between the baseline and post-smoking samples were assessed using linear modeling. Enrichment of previously published smoking-related gene-expression signatures and biologic pathways were determined using Gene Set Enrichment Analysis (GSEA).

Results: We identified 91 genes differentially expressed between the baseline and post-smoking samples ($FDR < 0.25$) related to innate immunity, the cytokine response, xenobiotic metabolism, and oxidative stress. Furthermore, we found concordance between the genes induced by ASE *in-vivo* and *in-vitro* with human bronchial epithelial cells on an air-liquid interface. Additionally, genes changing with ASE were enriched among genes that we previously identified as changing with chronic smoke exposure ($GSEA FDR < 0.05$). While many genes altered by ASE are altered similarly in chronic smokers, chronic smokers have low expression of metallothionein genes despite induction of metallothionein expression by ASE both *in vivo* and *in vitro*.



Conclusions: Acute exposure to as little as three cigarettes induces gene-expression alterations in the bronchial airway epithelium within 24 hours that resemble changes seen in the airway of chronic active smokers and are likely to reflect the direct cellular response to tobacco smoke components. The difference in the short-term and long-term effects of smoking on metallothionein expression requires further study given these enzymes' role in responding to oxidative stress and metal detoxification.

7. TRANSCRIPTOMIC PROFILING OF BRONCHIAL BIOPSIES AMONG MILITARY AND VETERAN PERSONNEL WITH SUSPECT LUNG CANCER: THE DECAMP CONSORTIUM

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Introduction: The Detection of Early Lung Cancer Among Military Personnel Study (DECAMP) is a multicenter consortium aimed at improving lung cancer screening and diagnosis via imaging and molecular biomarkers measured in minimally invasive and noninvasive biospecimens. Bronchial biopsies, one type of collected biospecimen, provide an opportunity to characterize the airway immune response in smokers who develop lung cancer compared to those that do not. In this study, we leveraged this unique cohort to identify differences in gene expression from bronchial biopsies of smokers with and without lung cancer.

Methods: We performed total RNA sequencing on 44 fresh-frozen bronchial biopsies from the right upper lobe carina of individuals enrolled in DECAMP-1 with (n=21), and without (n=16) lung cancer and of individuals pending a definitive diagnosis (n=7) of lung cancer.

Results: Six samples failed quality control parameters and 8 samples were removed because of uncertain lung cancer diagnosis or smoking history. Among the remaining 30 samples (n=20 with and n=10 without lung cancer), subjects with no cancer were significantly older and had a greater proportion of former smokers (p<=0.02). 104 pathways that are differentially expressed in smokers with lung cancer vs smokers without lung cancer (FDR< 0.05) using a linear model correcting for smoking status. Pathways downregulated in lung cancer subjects were associated with immune activation, inflammatory response, chemotaxis, and cytokine production.

Conclusions: These preliminary results on a small set of subjects suggest that there is an immunosuppressive microenvironment in the airway field of injury of smokers with lung cancer. Sequencing samples from a larger number of subjects combined with immunohistochemistry validation on adjacent paraffin-embedded airway tissue is needed to confirm these observations. Given the success of



immunotherapy in treating lung tumors, we hope that these insights will provide evidence that similar agents may be beneficial in preventing and treating early-stage disease.

8. AN HHIP MODEL FOR UNCOVERING MECHANISTIC CAUSES OF COPD

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Background: Chronic Obstructive Pulmonary Disease (COPD) affects over 12 million people and is the third leading cause of death in the U.S. Current treatments target symptoms but a failure to understand the molecular mechanisms underlying COPD precludes the development of new, targeted therapies. Genome Wide Association Studies (GWAS) show HHIP is associated with COPD. Mouse models confirm a vital role for HHIP in lung development and response to injury. Longitudinal studies show approximately half of all individuals with COPD fail to reach a normal level of lung function by middle age, implicating a role in lung development in COPD. Our objective is to determine mechanisms by which HHIP and regulation of hedgehog signaling affects lung development, response to injury, and COPD pathogenesis.

Methods: Human induced pluripotent stem cells (hiPSCs), an important tool for modeling human development and genetic diseases, were differentiated into lung progenitors expressing NKX2.1, the earliest known marker of the lung epithelium. An iPSC line with a NKX2.1/GFP reporter was used to determine efficiency of specification and sort cells for RT-qPCR analysis. The CRISPR/Cas system was used to target HHIP and create haplo-insufficient and knockout cell lines. The effects of perturbing hedgehog signaling, with the HHIP-targeted cell lines and small molecules, on lung progenitor specification were characterized.

Results: Conditions with increased Hedgehog activity resulted in less efficient expansion and/or specification of NKX2.1+ progenitor cells. NKX2.1+ lung progenitors were further differentiated and exposed to cigarette smoke condensate and evidence of cigarette smoke specific injury was observed.

Conclusion: HHIP may be an important regulator of lung specification and this in vitro platform provides an exciting opportunity to find and characterize mechanisms responsible for COPD pathogenesis.

9. L2PB1 CELLS ARE ESSENTIAL FOR THE INHIBITION OF 3D CELLS BUT NOT SPLENOCYTES

TUMOR SPHEROIDS BY SYNGENEIC PERITONEAL IMMUNE

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Introduction: Programmed Death Ligand 2 positive B1 (L2pB1) cells have a unique immunoglobulin repertoire that is poly-reactive to self-antigens. The active accumulation of L2pB1 cells inside tumor in our animal model led us to hypothesize that this subpopulation of B1a cells may play roles in the immunosurveillance of cancer. Here, we report our investigation of the role of L2pB1 cells in the antitumor response using a three dimensional (3D) murine melanoma model. Our results showed that depletion of L2pB1 cells rendered the loss of tumor-inhibition effects of the syngeneic peritoneal immune cells.



Methods: Lymphocytes were collected from L2pB1 cell depleted and non-depleted peritoneal cavity wash (PCW) from an inducible knockout mouse model. 3D Tumor spheroids were incubated with syngeneic PCW cells or splenocytes. Spheroid cross sectional area (CSA) and volume were measured and analyzed using a Celigo plate imager.

Results: Tumor spheroid growth was significantly inhibited following incubation with syngeneic PCW cells but not splenocytes. Depletion of L2pB1 reverted the tumor-inhibition effect and showed negligible difference from the untreated control and the splenocyte-treated group. This indicates that L2pB1 cells are essential for the tumor-inhibiting effects of the autologous peritoneal immune cells.

Conclusion: Our results suggested that immune cells from the peritoneal cavity but not spleen have antitumor functions. In particular, peritoneal L2pB1 cells play an essential role in cancer inhibition. Future studies of L2pB1 cells and their anti-tumor functions could result in a novel immunotherapy of cancer.

10. IPSC-BASED MODELING OF HEREDITARY FORMS OF COLORECTAL CANCER (CRC)

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Background: Colorectal cancer (CRC) is the second leading cause of cancer-related death in the USA. During 2017 it is estimated that one million people will manifest the disease. Among them, 30% of the patients will have hereditary forms of CRC. Familial adenomatous polyposis (FAP) and Lynch syndrome (LS) are two major of these hereditary forms. They are caused by mutation of the Adenomatous Polyposis Coli (APC) tumor suppressor gene and the DNA mismatch repair (MMR) genes, respectively.

Objectives: To recreate human intestinal tissue from directed differentiation of human induced pluripotent stem cells (iPSC), providing a model to study early carcinogenic events and compare the molecular pathways involved in both genetic forms of the disease.

Hypothesis: Differences between patients and healthy individuals will allow us to describe their molecular profiles and look for new biomarkers of disease.

Methods: Samples from FAP and LS patients were reprogramed into iPSCs. In addition, gene editing was used to create an isogenic platform to compare APC+/+ vs APC+/- cells. Pluripotent cells were first induced to differentiate into definitive endoderm and then pushed towards mid hindgut and intestinal fate using optimized growth factor cocktails. Monolayer cultures containing positive cells for intestinal markers developed into spheroidal precursors of human intestinal organoids (HIOs). HIOs were collected and matured in 3D matrigel cultures in the presence of intestinal factors and analyzed by morphology, IHC and expression profiles.

Results: We have found significant differences on gene expression profiles related to cell proliferation and Wnt target genes. Going in depth with these findings could shed light on disease mechanisms of action.

Conclusions: Our study open the door towards future applications in high-throughput drug screening and gene therapy.



11. MULTIPLEXING METABOLOMIC-BASED DISEASE DIAGNOSIS BY SERS PLATFORM

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Background: Surface-Enhanced Raman Scattering (SERS) based platforms have the potential to be multiplexing, rapid, ease-to-use diagnostic tool for a wide variety of human health concerns. SERS has been demonstrated as an ultrasensitive method to detect and identify molecules at low concentrations without the need of fluorescent labeling. SERS arises from the well-known Raman cross-section enhancement effect on molecules close to (< 5 nm) the surfaces of nanostructured metal substrates. Since Raman vibration features are unique dependent to the molecular structure, SERS has been shown to be a sensitive and specific method for studying biochemical activity near cell membranes and in the extracellular region. These attributes allow SERS to be a cell-growth free, label-free, sensitive and specific disease diagnostic platform. Our work will demonstrate the potential of SERS for diagnostic for urinary tract infection (UTI), sexually transmitted diseases and cancer.

Methods: The nanoparticle-covered SERS active substrate used in all the experiments are synthesized in our laboratory and had been characterized for its sensitivity and specificity to distinguish different bacteria base only on their spectral features. The causative agents for each disease, either clinically isolated or commercially available, are cultured in our laboratory. We design sample processing and enrichment procedure to harvest and isolate each agents from culture and acquire SERS spectrum. A 785 nm excited laser Raman spectrometer (Renishaw®) are used for all the SERS experiment.

Results: We obtained high-quality SERS spectra for twelve clinically-isolated UTI-causing bacterial strains with pre-determined antibiotic susceptibility profile that are grown in spiked urine to clinically relevant concentration, and identify the biomarker for each bacteria strain from their SERS spectra. We implement a machine-learning algorithm for classification these twelve bacteria strains and to predict the strain identify in an unknown sample. We also obtain SERS spectra on gold and silver substrate for the intracellular parasite *C. trachomatis* and the fastidious *N. gonorrhoeae*, the causative agent for the sexually transmitted diseases chlamydia and gonorrhea respectively. We decipher the biomarker for these two bacteria from their SERS spectra and understand how these biomarkers related to the different lifestyles of the two bacteria. Finally, we will briefly discussed the ability for SERS to distinguish a breast cancer cell line grown *in vitro* from normal cell based on their time-dependent change of SERS spectral features.

Discussion: With the exception of *C. trachomatis*, whose key SERS biomarker are determined to be protein molecules on their cell membrane, the biomarker for all bacteria strain as well as for cancer cell are determined to be purine metabolites: adenine, hypoxanthine, xanthine, guanine, uric acid, AMP and guanosine. Purine metabolites are secreted by the cells to the surrounding environment as response to starvation. The difference in the relative concentration of each of the purine metabolite, results from the presence/absence of enzymes in the purine metabolism pathway and the difference in their activity, in the SERS spectrum serve as the intrinsic, highly specific molecular biomarker for the bacteria strain. In addition, the time-dependent change of the relative contribution of the purine metabolites in a bacteria strain can be used as additional revenue for diagnostic. Our SERS results also demonstrate SERS can be used as powerful bioanalytical tool to study near cell membrane activity and biochemical process in bacteria under stressful (i.e. starvation) condition more generally.

Conclusion: We demonstrate that SERS has the potential to be a rapid, growth-free, sensitive and specific for the diagnosis of UTI, chlamydia and gonorrhoeae, and cancer. When combined with *a priori* developed reference library with antimicrobial susceptibility, portable instrument and effective sample processing and enrichment procedure, SERS has the potential to be in-clinic diagnostic method in which physician will be able to obtain result in less than one hour. Our work with chlamydia and gonorrhea also demonstrate the

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potential of SERS to be a powerful bioanalytical probe to study near cell membrane activity and biochemical process in bacteria more generally.

12. HIV/ ANTIRETROVIRAL THERAPY ASSOCIATED INSULIN RESISTANCE: A POSSIBLE ROLE OF COMPLEMENT SYSTEM.

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Background: Anti-retroviral therapy (ART) has significantly increased life spans and reduced the incidence of AIDS globally. However, incidence of HIV associated metabolic syndrome has steadily increased, the molecular basis for which is unknown. The adipose tissue is a strong link between chronic inflammation and metabolic syndrome through the production of several peptide hormones and cytokines collectively called Adipokines. The complement proteins, especially C3, is one such Adipokine that has been implicated in insulin resistance and abnormal lipid metabolism. HIV activates complement system. HIV proteins like nef and gp41 have been shown to induce the production of complement proteins C3 in monocytes derived macrophage, astrocyte and neurons associated with AIDS associated neurological disorder.

Objectives: This study aims at determining the role of HIV mediated complement induction and activation in development of insulin resistance and lipid dystrophy.

Methods: Subcutaneous white adipose tissue from bariatric surgery patients were lysed in HNE lysis buffer and ELISA was done to measure level of complement proteins C3 and C3a Linear regression analysis was done to determine correlation between levels of C3 and C3a with BMI. For adipogenesis studies, human adipose stem cells (ASCs) were differentiated in vitro and western blot, ELISA was done to determine levels of markers of adipogenesis.

Results: Here we present some preliminary data that forms the rationale for our hypothesis and experimental design. We observe a linear correlation between C3/C3a with BMI. We also describe a method of differentiating human ASCs into adipocytes in vitro, for studying the effect of HIV infected monocytes on adipogenesis and adipocyte function.

Conclusions: This study will help us understand the molecular mechanism underlying HIV infection and antiretroviral therapy associated co-morbidities like lipid dystrophy and insulin resistance. We will also evaluate plasma complement induction and activation as a biomarker for HIV/ ART associated diabetes.

13. THYROID CONVERSION OF MOUSE ESC-DERIVED ANTERIOR FOREGUT THROUGH TRANSIENT OVEREXPRESSION OF NKX2-1

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Background: This project seeks to improve our knowledge of the thyroid specification process. Thyroid cells are derived from mouse embryonic stem cells (mESCs) by directed differentiation through multiple intermediate developmental stages, including anterior foregut endoderm (AFE), prior to Nkx2-1+ thyroid progenitor specification.

Methods: To investigate if transient Nkx2-1 expression can increase the efficiency of thyroid specification, we utilized a mESC line double knock-in GFP-T/hCD4-Foxa2 with a doxycycline inducible (Tet-On) Nkx21 transgene.

Results: Transient activation of the Nkx2-1 transgene at the AFE stage leads to stable induction of high levels of endogenous Nkx2-1 as well as early and mature thyroid-specific markers including Pax8, Foxe1, Tg, Nis, and Tshr. These cells mature and organize into follicle-like structures in 3D culture. Critical determinants of thyroid lineage specification have been revealed by variations in developmental stage timing, signaling pathways, and sorting of AFE-stage subpopulations. To provide further insights into the mechanisms of thyroid specification, RNA-Seq data acquired from relevant stages has identified potential genes involved in early thyroid development.

Conclusion: The results demonstrate that Nkx2-1 can act as a stage-specific inductive signal during directed differentiation of mESCs to thyroid follicular cells. This method has provided novel insights into thyroid specification and provides an efficient system for deriving and studying thyroid cells, which can be used for in vitro modeling of development and disease.

14. GLIADIN DEGRADATION AND EPITOPE ABOLISHMENT IN THE GASTRO-INTESTINAL TRACT OF MICE

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Introduction: Celiac disease is a chronic immune-mediated inflammation of the duodenum, triggered by gluten contained in wheat, barley and rye. Our previous studies have shown that *Rothia*, Gram-positive oral bacteria, have the ability to degrade and detoxify gluten *in vitro*. The objective of this study was to test if *R. aeria* bacteria can degrade gluten that is naturally contained in mice food *in vitro* and *in vivo*.

Methods: Gluten digestion *in vitro* was monitored in mice chow with and without added *R. aeria* bacteria (OD₆₂₀ 200 per 1 g of chow) after 0, 2 and 4h incubation. For the *in vivo* experiment, balb/c mice were fed with chow with and without added *R. aeria* bacteria and sacrificed after 2 h. The stomach, duodenum, jejunum and ileum contents were harvested and gluten degradation was assessed by SDS-PAGE and immunoblotting, and with the R5-ELISA assay detecting immunogenic gluten epitopes.



Results: *In vitro*, gliadins were stable in food incubated in the absence of *R. aeria*, but degraded within 2h in the presence of *R. aeria*. In vivo, fasted mice readily consumed the 1 g food without or with the added bacteria. After 2h, the distribution of the chyme in the stomach and the small intestines was determined to be approximately 64%/36%. Gliadins were detected by immunoblotting in the stomach, but not in the small intestinal samples, of both the control and the *R. aeria*-fed mice. Gliadin quantitation and ELISA results provided evidence that gliadins and epitopes were reduced by 20 % and 32.6 % as compared to the control mice, respectively.

Conclusion: These study results provide the basis for further exploring *R. aeria* as a first probiotic candidate to aid in the digestion of immunogenic gluten epitopes *in vivo* to benefit the celiac disease patient population.

15. IMPACT OF DIESEL ENGINE EXHAUST EXPOSURE ON THE AIRWAY TRANSCRIPTOME

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Introduction: Recent epidemiological studies show that Diesel Engine Exhaust (DEE) exposure is associated with lung cancer, however the mechanism by which this occurs is not well understood. The goal of this study was to assess transcriptomic alterations in nasal epithelium of DEE exposed factory workers to better understand the physiologic effects of DEE.

Methods: Nasal epithelium brushings were obtained from 41 subjects who work in a factory with DEE exposure, and 38 comparable control subjects who work in factories without DEE exposure. The median Elemental Carbon (EC) levels of exposed individuals was 60.7 $\mu\text{g}/\text{m}^3$, with a range of 17.2-105.4 $\mu\text{g}/\text{m}^3$, respectively. RNA was isolated from nasal epithelial cells, and profiled for gene expression using Affymetrix microarrays. Linear modeling was used to detect differential expression between DEE exposure and controls. Pathway enrichment in differentially expressed genes was assessed using EnrichR. Boxplot analysis was used to investigate the interaction effect model.

Results: We found 225 genes whose expression is associated with DEE exposure at FDR $q < 0.25$, after adjusting for smoking status. Within this set of genes, we observed increased expression of oxidative stress response, cell cycle, and protein modification genes, as well as decreased expression of genes involved in transmembrane transport, such as CFTR. We also found 8 genes at FDR $q < 0.25$ that have altered expression in the DEE: smoking status interaction model, suggesting a synergistic relationship between the effects of these exposures on some aspects of the physiological response. For these genes, the effects of DEE were generally more dramatic in never smokers.

Conclusions: The transcriptomic alterations we identified may help provide insight into the underlying mechanisms of DEE carcinogenicity.

16. META-OMICS: COMPARING GENOMIC AND



TRANSCRIPTOMIC ANALYSES OF GUT MICROBIOMES IN A CONTROLLED DIET STUDY

TYLER FAITS¹, MANI SOLAIAPPAN¹, ALICE LICHTENSTEIN², ANNE KANE³, W. EVAN JOHNSON¹

Background: 16S ribosomal DNA sequencing has made metagenomic studies of the human microbiome cheap and reliable, and analytic tools such as Qiime have made analysis of these data straightforward and reproducible. Sequencing of whole microbial RNA provides additional information about species' activity level and metabolic function, but analytic software for such experiments is underdeveloped.

Methods: We created a metatranscriptomic analysis pipeline, and compared whole RNA-seq to 16S rDNAseq data in a controlled diet study. Eleven human subjects were each placed on three diets with distinct carbohydrate compositions: simple sugars, refined carbohydrates, and unrefined/complex carbohydrates. Subjects remained on each diet for five weeks; whole RNA and 16S rDNA was isolated from fecal samples collected at the end of each diet.

Results: Relative abundance of taxa varied wildly between subjects, but showed no significant change based on diet. Microbial metabolic activity was much more robust between individuals, indicating that disparate taxa may perform similar ecological roles. We also found that species with low abundance in samples can show high transcriptional activity, implying that an organism need not be dominant in order to shape the biochemical environment.

Conclusion: Metatranscriptomic analyses may recapture many results found in metagenomic studies, but offer additional metabolic information.

17. POSTPRANDIAL OXYTOCIN LEVELS AND HEDONIC FOOD INTAKE, REWARD SENSITIVITY, AND COGNITIVE CONTROL IN YOUNG FEMALES WITH LOW WEIGHT EATING DISORDERS AND HEALTHY CONTROLS: RATIONALE, STUDY DESIGN, AND BASELINE PARTICIPANT DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

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Background: Low weight eating disorders (LWED) are characterized by extremes in food consumption and eating-related behaviors resulting in low body weight. Extremes in food intake are assumed to arise from a dysbalance between two brain circuits, i.e., the reward circuit determining the hedonic value of food and the cognitive control network suppressing behavioral urges. However, the pathophysiological mechanisms underlying LWED are mostly unknown. Our prior work demonstrated an association between serum oxytocin levels and severity of eating disorder psychopathology in individuals with LWED when compared with health controls (HC). We hypothesize that pathways involving the anorexigenic hormone oxytocin impact reward sensitivity and cognitive control, and play a key role in the pathophysiology of LWED. Here we provide information on the rationale, trial design, and baseline data for our current study designed to investigate the relationship between postprandial oxytocin levels and hedonic food intake,



reward sensitivity, and cognitive control in females as a potential pathophysiological mechanism underlying dietary restriction in LWEDs.

Methods: We enrolled a cohort of healthy (n=20) and LWED (n=40) females ages 10-21 years in an observational prospective 18-month NIMH R01 study. At baseline, we tested LWED individuals and HC with regards to eating psychopathology, postprandial serum oxytocin levels following a standardized meal, hedonic food intake (Cookie Taste Test), reward sensitivity (Power of Food Scale), and cognitive control (Go/NoGo task).

Results: Within and between-group statistics for demographic and clinical characteristics will be presented. In LWED individuals, compared to HC, we predict (a) higher postprandial oxytocin levels, (b) reduced hedonic food intake, (c) reduced reward sensitivity, and (d) excessive cognitive control.

Conclusion: Results from this study will provide insights into the psychopathology of LWED, further advancing treatment strategies for this population.

18. AN iPSC-BASED UNIVERSAL GENE CORRECTION STRATEGY FOR TREATING FAMILIAL TRANSTHYRETIN AMYLOIDOSIS

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Introduction: Familial transthyretin amyloidosis (ATTR) is an autosomal dominant protein folding disorder resulting from over 100 possible different substitution mutations in the transthyretin (TTR) gene. Disease pathology and patient survivability greatly vary depending on which mutation a patient inherits, with an average time of diagnosis to death of only 5 to 10 years. Currently, the only approved treatment for ATTR is liver transplantation; however, not all patients are eligible for surgery and donor organ shortages necessitate alternative approaches. To meet this need, we have utilized transcription activator-like effector nuclease genome editing to develop a universal gene correction strategy for ATTR.

Methods: Our laboratory has recently generated an extensive, genetically diverse library of ATTR patientspecific induced pluripotent stem cells (iPSCs). Using iPSCs derived from a patient with the most proteotoxic TTR variant, we have successfully introduced a fluorescently labeled, wild-type version of TTR into exon 1 of endogenous mutant TTR. "Corrected" iPSCs were directly differentiated into hepatocyte-like cells and supernatant was analyzed via mass spectrometry (MS) for secretion of wild-type and mutant TTR.

Results: MS analysis of supernatant of directly differentiated hepatocytes derived from corrected iPSCs showed complete ablation of amyloidogenic TTR, while levels of wild-type protein remained unperturbed.

Conclusions: To determine the effectiveness of our gene correction strategy, supernatant from corrected hepatocytes will be co-incubated with iPSC-derived neurons and cytotoxicity will be determined via Annexin V staining. The present study provides proof-of-concept for an iPSC-based universal gene correction strategy for treating a complex, multisystemic disorder. Moreover, as genetic correction of the ATTR disease-specific iPSC line introduced a fluorescently labeled TTR, the resulting reporter line also serves as a tool to study TTR production in real-time, granting us the opportunity to interrogate the protein's role in additional cell types and conditions.



19. GENEHIVE: A HIGHLY FLEXIBLE DATA WAREHOUSE FOR THE STORAGE AND ANNOTATION OF HIGH-THROUGHPUT DATA

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Background: High-throughput technologies such as expression microarrays and RNA sequencing are powerful tools for translational researchers, as they show the biological consequences of experimental perturbations, disease states and genetic manipulations. However, the logistics of storing, securing, and sharing genome-scale data within a conventional file system are challenging, often resulting in unnecessary duplication or sequestration of data. Many investigators have addressed these issues with a patchwork of solutions, often storing raw data in third-party cloud storage and placing sample annotation in unversioned, proprietary formats (e.g., Excel files). Furthermore, such data are often transmitted through insecure channels such as public servers, email, or portable hard drives or flash memory devices. Moreover, locating data associated with a given study and reproducing results are challenges frequently faced after trainees associated with such work have moved on. In response to these concerns, we developed GeneHive, a secure, API-driven data warehouse that allows for the storage of raw experimental data and associated metadata in a highly flexible manner.

Methods: The GeneHive instance described here contains data structures corresponding to concepts such as samples, sample sets, microarray platforms, alignments, normalized data, and weighted feature sets (e.g., biomarkers). This infrastructure also ensures that records are referred to in an unambiguous, versioncontrolled manner. To facilitate the usage of GeneHive by computational researchers, we have also developed a software package for the R programming language to allow integration of GeneHive-based storage with existing workflows, as well as a graphical interface for investigators without the expertise or need to use R.

Results: These capabilities are now being used by the BUSM Microarray Core and Section of Computational Biomedicine to store, organize, retrieve and share genome-scale data in a secure manner.

Conclusion: GeneHive is a highly flexible, structured resource for storage of genome-scale data. The open-source nature of GeneHive will also allow other groups such as CTSI hubs to readily adopt this platform for local use.

20. PARENTAL SOCIO-CONTEXTUAL FACTORS ASSOCIATED WITH SELF-HARM AND SUICIDAL BEHAVIOR FOR ASIANAMERICAN WOMEN

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Instruction: Asian-American adolescent girls have the highest rates of depression of all racial/ethnic and gender groups (NAMI, 2011). Research has linked "disempowering parenting styles" (Abusive, Burdening, Culturally-Disjointed, Disengaged, and Gender-Prescriptive parenting) to self-harm and suicidality among young Chinese, Korean, and Vietnamese women (Hahm et al., 2014). While evidence suggests that most Asian-American parents do not employ harsh parenting styles (Kim, Wang, Orozco-Lapray, Shen, and



Murtuza, 2013), our 2014 study suggested that disempowering parenting styles were prevalent in the homes of Asian-American women who were classified as high-risk for suicidality and self-harm behaviors.

Methods: The current study analyzes the data of 16 daughters of Asian immigrants reporting a history of these behaviors in order to better understand the experiences of immigrant parents utilizing disempowering parenting strategies. This study uses a thematic analysis methodology, which seeks to identify, analyze, and report patterns or themes within qualitative data. Two coders read through the interview transcripts, listened to the audio recordings, and used NVivo to code for examples of disempowering (ABCDG) parenting and marked references to parent backgrounds.

Results: Results suggest that there are 7 major socio-contextual predisposing parental themes associated with the formation of ABCDG parenting: (1) mental and physical health concerns, (2) marital discord, (3) sociocultural linguistic barriers, (4) job-related stress, (5) fragile support networks, (6) trauma from the country of origin, and (7) the vague transmission of personal history. Behind each daughter's pathology were the collective struggles of both these young women and their parents, rooted in complex socio-cultural inequalities. Interventions are needed to address the social, contextual, and systemic factors that influence immigrant family systems.

Conclusions: Findings suggest there is an urgent need for collaborations among researchers, Asian community organizations, and policymakers to adequately protect the lives of young Asian-American women.

21. THE FEASIBILITY AND EFFICACY OF A MENTAL AND SEXUAL HEALTH INTERVENTION FOR 1.5 AND 2ND GENERATION CHINESE-, KOREAN-, AND VIETNAMESE-AMERICAN WOMEN: A RANDOMIZED CONTROLLED TRIAL

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Instruction: Recent epidemiological data document two emerging public health problems among Asian-American women in the United States (US): high suicide rates and a growing rate of HIV/AIDS diagnoses. To our knowledge, Asian Women's Action for Resilience and Empowerment (AWARE) is the first gender and culture specific intervention designed for Chinese-, Korean-, and Vietnamese-American young women with histories of interpersonal trauma. To test whether AWARE reduced depressive symptoms and sexual risk behaviors in participants over time. We also tested whether treatment effects would differ by the presence of a PTSD diagnosis at baseline.

Methods: We employed a two-arm randomized clinical trial. Of 435 women who were initially screened, 63 women were eligible and randomized into either the intervention (n=32) or waitlist control (n=31) group (14.3% inclusion rate). AWARE consisted of 8 group sessions. Retention rates, depressive symptoms, and sexual risk behaviors were assessed at baseline, post-intervention, and 3-month follow-up. The PCL-C (PTSD Checklist, Civilian version) was used to diagnose PTSD at baseline.

Results: 87.5% of randomized participants who started AWARE completed at least 6 of the 8 sessions. Compared to the control group, the intervention group did not experience reductions in depressive symptoms or sexual risk behaviors over time after controlling for baseline depressive symptoms. However, women with PTSD at baseline experienced significant reductions in depressive symptoms with an effect size of 0.84.



Conclusions: While AWARE did not demonstrate an overall effect on depressive symptoms or sexual risk behaviors, it is a highly feasible intervention that is efficacious in reducing depressive symptoms over time for those with PTSD. A full RCT is needed for future studies.

22. TLR2 LIGANDS DIFFERENTIALLY INCREASE CYTOKINE MRNA STABILITY BY HUR ACTIVITY

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Background: Sepsis is a complex syndrome characterized by an aberrant immune response, shock, and a mortality rate that has remained relatively unchanged for several decades. It remains a critical public health issue as the leading cause of ICU mortality and estimated 20 million cases per year, at a cost of 24 billion dollars annually.

The altered physiology in sepsis is thought to be due to dysregulation of inflammatory cytokines produced in response to infection by Gram-positive or Gram-negative bacteria. Studying the components of bacteria that provoke inflammation is likely to yield valuable insight into altered cytokine expression in sepsis. Lipoteichoic acid (LTA) is a cell wall component exclusive to Gram-positive bacteria that is the functional equivalent to lipopolysaccharide (LPS), the major cell wall component of Gram-negative bacteria. However, distinct patterns of inflammatory cytokine expression have been shown for LTA and LPS, and activation of their respective receptor pathways, TLR2 and TLR4. Inflammatory cytokines are upregulated at multiple levels of gene expression, including mRNA stability, through the actions of RNA binding proteins, such as HuR.

Although several studies have used LPS to induce changes in cytokine mRNA stability, the role of LTA in regulating mRNA stability remains unknown, along with the role of mRNA stability in sepsis mortality. The focus of this study was to determine the effects of LTA on cytokine mRNA stability.

Methods: The mouse macrophage cell line J774.2 as well as primary peritoneal macrophages were stimulated with LPS, LTA or the TLR2 ligand Pam3CSK4. Levels of cytokine TNF- α , IL-6, and IL-1 β mRNA were measured via real time PCR. mRNA stability was calculated using Actinomycin D treatment. The RNA binding protein, HuR, was immunoprecipitated from cell extracts and assessed for binding to cytokine mRNA after stimulation. Knockdown with siRNA targeting HuR was performed followed by mRNA stability.

Results: Both LPS and LTA induced all three cytokine mRNAs, and half-life analysis showed an increased in the stability as well in primary macrophages. IL-6 and IL-1 β mRNA stability was increased but not TNF α in J774.2 cells. The effect of Pam3CSK4 on mRNA stability was more pronounced in J774.2 cells. HuR binding to cytokine mRNAs correlated with the increase in mRNA stability, and siRNA knockdown decreased the stabilizing effect of either TLR ligand on IL-6 and IL-1 β , but not TNF- α .

Conclusion: We demonstrate the novel finding that TLR2 ligands regulate different cytokine mRNA expression at the post-transcriptional level through HuR activity.



23. MODELLING AATD LUNG DISEASE USING ALVEOLAR EPITHELIAL CELLS DERIVED FROM GENE-EDITED IPSC

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Introduction: Type II alveolar epithelial cells (AEC2) play key roles in the response of lung epithelial cells to injury and the pathogenesis of lung disease. These cells are capable of producing alpha-1 antitrypsin (AAT) but little is known about their role in alpha-1 antitrypsin deficiency (AATD) pathogenesis. Mutation of the SERPINA1 gene leads to low levels of circulating AAT protein causing emphysema. We hypothesise that AEC2s play an intrinsic role in AATD pathogenesis and therefore we sought to engineer a novel *in vitro* model to enable the derivation of lung epithelial cells from patients with AATD.

Methods: We targeted fluorescent reporters into the endogenous NXK2-1 and surfactant protein C (SFTPC) loci of non-diseased human iPSC. Using a directed differentiation approach that recapitulates embryonic development we generated lung progenitors followed by putative AEC2s and characterization of these cells was carried out by RNA-seq, intracellular flow cytometry and ELISA for AAT, and electron microscopy for cellular ultrastructure.

Results: Distal alveolar epithelial cells (SFTPC+) iPSCs express AEC2-specific genes by RNA-seq, together with increased expression of AAT mRNA and protein, compared to undifferentiated cells. iPSC-derived SFTPC+ cells have ultrastructural characteristics of lamellar bodies and Western blotting confirms functional processing of SFTPB protein. We targeted a fluorescent reporter into the SFTPC locus of an iPSC line generated from an individual homozygous for the SERPINA1 Z-gene mutation (PiZZ) before CRISPR-based gene editing to correct the PiZZ genotype to syngeneic PiMM iPSC.

Conclusion: We find that differentiation of PiMM iPSC lines can generate AEC2-like cells which contain functional lamellar bodies similar to those found in mature AEC2s, and that these cells express AAT at significant levels at both mRNA and protein levels. We have also created gene corrected syngeneic clones from individuals with AATD for studies aimed at determining the role of AEC2s in AATD lung disease.

24. GENERATION OF MATURE HUMAN LUNG ALVEOLAR TYPE II CELLS FROM PLURIPOTENT STEM CELLS FOR USE IN DISEASE MODELING

ANJALI JACOB, BA

Introduction: Human tissues arising late in evolutionary time, such as lung alveoli unique to air breathing organisms, have been challenging to generate *in vitro* from pluripotent stem cells (PSCs) because there are limited lower organism model systems available to provide necessary developmental roadmaps. Since pulmonary alveolar epithelial type II cell (AEC2) dysfunction has been implicated as a primary cause of pathogenesis in many poorly understood lung diseases that lack effective therapies, including idiopathic pulmonary fibrosis and COPD, generating AEC2s *de novo* via directed differentiation of PSCs would provide novel opportunities to study normal AEC2 development as well as the pathogenesis of monogenic alveolar diseases.

Methods: We differentiate human PSCs into AEC2s, the facultative progenitors of lung alveoli. Using gene editing to engineer multicolored fluorescent reporter PSC lines (NKX2-1^{GFP}; SFTPC^{tdTomato}), we track and



purify human SFTPC+ alveolar progenitors as they emerge from NKX2-1+ endodermal developmental precursors. We also use Crispr technology to gene-correct a surfactant deficient patient iPSC line and model the patient's disease in iPSC-derived AEC2s.

Results: Purified PSC-derived SFTPC+ cells self-renew and display canonical AEC2 functional capacities, including innate immune responsiveness and the production of lamellar bodies able to package surfactant. Guided by time series RNA-sequencing, we find that AEC2 maturation involves downregulation of Wnt signaling activity, and that the highest differentially expressed transcripts in the resulting SFTPC+ cells encode genes associated with lamellar body and surfactant biogenesis. Finally, we apply this novel model system to generate patient-specific AEC2s from iPSCs made from children carrying surfactant mutations, and we employ gene editing to observe that correction of the SFTPB^{121ins2} mutation restores surfactant processing in the cells responsible for their disease.

Conclusions: Taken together, these results show a novel approach for studying alveolar development, modeling alveolar disease, and future functional regeneration of a cell type unique to air-breathing organisms.

25. THE EFFECT OF BONE MARROW ON THE MICROENVIRONMENT OF THE HUMAN PANCREATIC ISLET: A PROTEIN PROFILE APPROACH

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Abstract: Stem cells are a new therapeutic modality that may support the viability and function of human organs and tissue. Bone marrow, containing multiple adult stem cells classes, was co-cultured in vivo and in vitro due to their supportive role in pancreatic beta cells. This paper examines whether bone marrow creates a microenvironment that supports human pancreatic islets in vitro by evaluating 107 proteins in culture media with mass spectrometry. The concentrations of each protein were measured in islet cocultured with bone marrow; this concentration was compared to the protein concentration in islet alone. Proteins were considered up- or down- regulated if p-values < 0.05 and fold change was greater than 2fold. In addition, proteins identified that were uniquely found in islet co-cultured with bone marrow, but not in islets or bone marrow only. A 95% protein probability was used as a threshold. Twenty-three proteins were upregulated, and sixteen proteins were downregulated. The function of each protein is listed based on the protein database, which include structural proteins (9 upregulated, 4 downregulated); antiprotease and anti-endopeptidase enzymes (8 upregulated); cation binding proteins (6 up-regulated). Six proteins were uniquely identified in islet co-cultured with bone marrow. Three are anti-proteases or antiendopeptidases, and one is a structural protein. These findings support the role of BM in creating a microenvironment that maintains human islet function and survival.

26. PREDICTORS OF SOCIAL SUPPORT AMONG NEWLY DIAGNOSED BREAST CANCER PATIENTS SEEKING CARE AT AN URBAN SAFETY NET ACADEMIC MEDICAL CENTER

NAOMI Y KO, MD MPH, KATE FESTA, CHRISTINE GUNN, SHARON BAK, NA WANG, KERRIE NELSON, JOHANNA FLACKS, SAMANTHA MORTON, TRACY A. BATTAGLIA

Background: Disparities in breast cancer are a worsening problem, requiring interventions that seek to address the delivery of high quality cancer care. Evidence from interventions routinely identify lack of social



support as one of the biggest barriers to care. This study explores predictors of social support in a diverse population of patients.

Methods: This is a secondary analysis of preliminary data from participants enrolled in Project SUPPORT (a randomized controlled comparative effectiveness trial designed to evaluate the impact of patient navigation with or without legal support and services) among women diagnosed with breast cancer between 2014-2016. Upon enrollment we administered the Medical Outcomes Survey (**MOS**) of social support to all participants. This validated survey measures functional support using an overall score (range 0-95) and 4 distinct domains: Emotional/Informational, Tangible, Affectionate and Positive Social Interaction. Using chisquared and t-tests we compared MOS scores across socio-demographic variables: age, race, language, insurance, health literacy and marital status.

Results: Of the 103 participants, mean age is 54.5 (SD = 10.6); 56% Hispanic, 19% Black, and 22% White and 2% Other; the majority had public insurance 76%; 66% speak English, 21% Spanish and 13% Haitian Creole. Only 36% have adequate health literacy. Only 32% are currently partnered. The overall mean total score for social support is 75.8 (+/- 23.6), median of 78.9 (range 60.5 – 98.7). Non-White participants scored significantly lower across all domains (mean overall MOS score 73.3 +/- 2.6) when compared with Whites (mean overall MOS score 84.5 +/- 4.8, p value = 0.04).

Conclusion: This is the first study to describe social support scores (overall and specific domains) from the validated MOS survey tool among a racially diverse, urban cancer patient population. We found significant differences by race. Studies to identify risks for low social support can help inform future targeted interventions.

27. FEASIBILITY AND ACCEPTABILITY OF THE “TAKE ACTION” PROGRAM FOR BREAST CANCER SURVIVORS

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Introduction: Breast cancer survivors often report persistent challenges with occupational engagement after cancer treatment (Lyons et al., 2015; Cheville & Tchou, 2009; Cheville et al., 2017), with as many as 66 percent of survivors reporting at least one long-term adverse sequelae (Cheville & Tchou, 2009). This study sought to investigate whether an occupation-based self-management program is feasible and acceptable among a low income, urban safety net breast cancer population.

Methods: A pilot study of the “Take Action” program was delivered within an urban, safety net hospital. Three cohorts of five Stage I-III breast cancer survivors were enrolled. “Take Action” consists of 6 sessions informed by Social Learning Theory, Problem Solving Therapy and the Person-Environment-Occupation Model. Feasibility was assessed by examining descriptive statistics regarding participant recruitment, adherence to group sessions, and successful data collection. Acceptability was assessed by retention, a satisfaction survey, and qualitative interviews.

Results: Regarding feasibility, 41 of 146 screened women were eligible (28%), of whom 15 enrolled (37%). Among those enrolled, 11/15 (73%) were retained and 4/15 dropped out (27%). Of the 11 retained, successful data collection was completed for 5 participants (45%). Regarding acceptability, the participants with complete data found the group and individual sessions to be highly beneficial (mean scores of 9.75 for both) and reported high satisfaction with the program (mean = 10).



Conclusion: The interim analyses suggest that “Take Action” may be both feasible and acceptable to implement. Our results will be informed by further investigation with qualitative analysis. The implementation of a patient-centered self-management program at an urban safety net hospital fills an important gap in the understanding of the unique needs in this vulnerable population. “Take Action” offers an important opportunity for occupational therapy to meet the needs of an underserved population.

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28. UNDERSTANDING AIRWAY AND VASCULAR SMOOTH MUSCLE CELL PHENOTYPES USING REPORTER-BASED PLURIPOTENT STEM CELLS AND CELL SHEET ENGINEERING

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Background: Angioplasty/stenting and bypass surgery have limitations as treatments for vascular occlusive disease, leading to the development of tissue-engineered blood vessels; however, immunogenicity remains a problem when autologous cell sources are unavailable. Induced pluripotent stem cells (iPSCs) provide an alternative cell source, where non-immunogenic cells of multiple lineages, including vascular smooth muscle cells (vSMCs), can be derived from patient-specific iPSCs. Although many studies have developed protocols for differentiating SMCs from iPSCs, there is no surface marker for isolating pure populations of vascular-specific SMCs. Smooth muscle actin (SMA) is a characteristic intracellular marker; however, it is also expressed in cardiomyocytes and myofibroblasts. Furthermore, SMA does not distinguish between SMCs from different anatomical locations (vascular, airway, etc.).

Methods: To address these issues, we have generated a novel mouse iPSC line containing a dual-reporter (green fluorescent protein (GFP) for SMA and red fluorescent protein (DsRed) for neural/glial antigen 2 (NG2)). We differentiated this line into vSMCs using PDGF-BB and TGF- β . vSMCs, which are characterized by co-expression of SMA/NG2, were isolated using our dual-reporter system. In addition, we have generated a human iPSC reporter line (eGFP for Acta2).

Results: iPSC-derived SMCs expressed characteristic markers (SMA, SM22 α , MHC, NG2), but with lower levels of expression compared to primary SMCs. Cell sheets were generated from iPSC-derived SMC-like populations (vascular (SMAGFP/NG2DsRed) and airway (SMAGFP)) using an enzyme-degradable hydrogel substrate, allowing for non-damaging release of cell sheets. The Acta2eGFP cells from the human iPSC system have been similarly assessed for gene expression.

Conclusion: We have developed reporter iPSC lines for deriving and purifying SMCs, and we have characterized the gene expression profile and functional profile of these cells. With this study, we will gain a better understanding of SMC phenotypes and their functional properties.

29. C-CBL EXPRESSION AS A NOVEL PREDICTIVE MARKER



OF SURVIVAL IN PATIENTS WITH METASTATIC COLORECTAL CANCER

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Background: Aberrant hyperactive Wnt/ β -catenin signaling is critical in colorectal cancer (CRC) tumorigenesis. Casitas B-lineage Lymphoma (c-Cbl) is a negative regulator of Wnt signaling, and functions as a tumor suppressor. The objective of this study was to evaluate c-Cbl expression as a predictive marker of survival in patients with metastatic CRC (mCRC).

Methods: Patients with mCRC treated at Boston University Medical Center between 2004 and 2014 were analyzed. c-Cbl and nuclear β -catenin expression was quantified in explanted biopsies using a customized color-based image segmentation pipeline. Quantification was normalized to the total tumor area in an image, and deemed 'low' or 'high' according to the mean normalized values of the cohort. A supervised machine-learning model based on bootstrap aggregating was constructed with c-Cbl expression as the input feature and 3-year survival as output.

Results: Of the 72 subjects with mCRC, 52.78% had high and 47.22% had low c-Cbl expression. Patients with high c-Cbl had significantly better median overall survival than those with low c-Cbl expression (3.7 years vs. 1.8 years; $p=0.0026$), and experienced superior 3-year survival (47.37% vs 20.59%; $p=0.017$). Intriguingly, nuclear β -catenin expression did not correlate with survival. No significant differences were detected between high and low c-Cbl groups in baseline characteristics (demographics, comorbidities), tumor-related parameters (primary tumor location, number of metastasis, molecular features) or therapy received (surgery, chemotherapy regimen). A 5-fold cross-validated machine-learning model associated with 3-year survival demonstrated an area under the curve of 0.729, supporting c-Cbl expression as a predictor of mCRC survival.

Conclusions: Our results show that c-Cbl expression is associated with and predicts mCRC survival. Demonstration of these findings despite the small cohort size underscores the power of quantitative histology and machine-learning application. While further work is needed to validate c-Cbl as a novel biomarker of mCRC survival, this study supports c-Cbl as a regulator of Wnt/ β -catenin signaling and a suppressor of other oncogenes in CRC tumorigenesis.

30. DETERMINING THE SIGNAL SOURCE IN MAGNETIC RESONANCE IMAGING OF MYELOFIBROTIC BONE MARROW

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Background: Magnetic resonance imaging (MRI) is a promising diagnostic method for evaluation of bone marrow (BM) myelofibrosis for offering non-invasive, wider-area coverage over conventional histology. Although the most striking feature in histology of myelofibrotic BM is the proliferation of reticulin fibers, the spectrum of findings involves abnormalities in cell number and composition, vessel proliferation, as well as possible pre-fibrotic abnormalities in matrix such as inflammation/edema. Correlation of MRI observations with such histological findings to accurately define the pathogenic phases of myelofibrosis has been



challenging. Here, we present the first MRI analysis of myelofibrosis in a mouse model (Gata-1^{low} mice) in an attempt to correlate MRI signal observations with histological findings.

Methods: T2-weighted MRI images of femur BM of Gata-1^{low} animals at different ages (12 to 46 weeks) showed high MR signal intensity, absent in age-matched wild-type controls, that is detected as early as 12 weeks of age, before onset of histologically-defined fibrosis in this mouse model. MRI acquisitions employing fat suppression, a technique that suppresses signals from lipids, together with the absence of chemical shift artifacts in the images, excluded the possibility of lipids as the source of the prominent MR signal. To determine the source of this prominent non-lipid signal in Gata-1^{low} BM, cellular composition, vascularization and vascular permeability were tested.

Results: Our findings demonstrate a multi-source nature of MRI signals in BM, which evolves from young (12 weeks) to old (40 weeks) Gata-1^{low} BM consistent with the pathologic progression of myelofibrosis, including increased microvessel density and fibrosis.

Conclusions: Our data in the GATA-1^{low} mouse model of myelofibrosis supports the importance of an integrative approach to further the development of this non-invasive modality for following the dynamics and progression of this multifactorial pathology in humans.

31. PLANAR CELL POLARITY IS NECESSARY FOR DERMAL WOUND HEALING

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Objectives: The orchestrated phases of inflammation, proliferation, and remodeling following injury are paramount determinants to the success of cutaneous wound healing. Wound healing recapitulates many aspects of development, whereby tissue structures are created through coordinated migration, proliferation and differentiation of cells. Although developmental pathways are reactivated in healing wounds, the role of Planar Cell Polarity (PCP), a morphogenesis pathway defining the proximal/distal sides of cells, remains relatively unexplored in this context. Since the PCP molecules Receptors tyrosine kinase like Orphan Receptors 1 (ROR1) and ROR2 are required for cellular migration and differentiation, we hypothesized that they would be essential to dermal healing. Further, we hypothesized that PCP signaling is dysregulated in patients with non-healing venous ulcers.

Methods: Wound closure following full-thickness dermal biopsy was determined in inducible ubiquitous ROR1 knockout (KO), ROR2 KO and ROR1/2 DKO mice and littermate controls. The PCP module was analyzed in C57Bl/6J wounds. FACS sorting of skin and lineage tracing studies in ROR2-lacZ reporter mice characterized the ROR positive cell. The signature of PCP components was determined in fibroblasts isolated from patients with non-healing venous ulcers and non-ulcerated skin.

Results: ROR1 and ROR2 are selectively expressed in fibroblast-like cells and their expression is induced by injury. ROR1 and ROR2 KOs exhibited delayed wound closure, and the ROR1/2 DKO provoked a further delay in healing. ROR2 KO wounds, but not ROR1 KO, expressed more markers of inflammation and fibrosis 7 days post wounding. Ulcer fibroblasts had reduced ROR2 compared to non-ulcerated cells, consistent with delayed healing in ROR2 KO mice.

Conclusion: These data suggest that PCP signaling modulates fibroblast function to coordinate wound healing through inhibition of inflammation and fibrosis. Our data support that ROR2 is a marker of a prohealing cell and suggests it as an attractive target for the clinical management of non-healing wounds.



32. A MEDIATOR OF TUMOR TO STROMAL CELL COMMUNICATION IN ORAL CANCER

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Introduction: Lysyl oxidase like-2 (LOXL2) is one of the members of lysyl oxidase family. LOXL2 is elevated in oral cancer and promotes metastasis. LOXL2 overexpression correlates with poor prognosis of oral squamous cell carcinoma. The objective of this study is to determine the effects and mechanism of a novel LOXL2 inhibitor (PXS-5033A) on the progression and invasiveness of oral squamous cell carcinoma in vitro and in vivo.

Methods: To define the target of PXS-5033A in oral cancer, its effects on human gingival fibroblasts which were influenced by tumor cell conditioned medium were investigated. Cell proliferation assays, signaling arrays and western blots were used to evaluate the effect of PXS-5033A on fibroblasts.

The effect of PXS-5033A on cancer growth and metastasis in vivo was investigated using an induced oral cancer mouse model by orthotopic injection of an aggressive oral cancer cell line into the tongues of immunodeficient mice. PXS-5033A at 10 mg/kg and 30 mg/kg were injected. Tumors were monitored by caliper measurements, and by in vivo imaging (IVIS). The mice were sacrificed after three weeks and their organs were subjected to immunohistochemistry staining for proliferation markers.

Result: LOXL2 inhibitor significantly inhibited gingival fibroblast proliferation triggered by tumor cell conditioned medium. LOXL2 inhibitor attenuated phosphorylation of PDGFRs P-PDGFR β Y771 and PPDGFR β Y857, but not PDGFR β Y751 in response to conditioned medium. PXS-5033A inhibited ERK 1/2 related signaling in fibroblasts and not AKT in response to conditioned medium. PDGFR activation by oral tumor cells is mediated possibly by PDGF-AB.

The data of caliper measurements, IVIS, and immunohistochemistry demonstrated that PXS-5033A significantly decreases oral cancer progression and metastasis in vivo.

Conclusion: LOXL2 enzyme is critical for oral cancer progression and metastasis. Inhibition of LOXL2 could provide strategies to develop therapeutic drugs for the treatment of oral cancer.

33. DEVELOPMENT OF AN EBOLA VIRUS PATHOGENESIS MODEL USING IPSC-DERIVED HEPATOCYTES

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Background: Ebola virus (EBOV) infection is known to cause severe human disease. Upon infection of the liver, measurable viral titers spike, and high viremia is associated with negative outcomes for patients. Therefore, we believe that the liver is a critical site for the successful life cycle of EBOV during human



disease and potentially a good target for therapeutic interventions. Primary human liver samples are difficult to acquire, and animal models of EBOV infection either incompletely recapitulate disease or are costly. Our goal is to use iPSC-derived hepatocytes to create an easily reproducible system for modeling human EBOV infection and pathogenesis.

Methods: We differentiated hepatocytes from human iPSCs using a previously published, step-wise protocol. iPSC-derived hepatocytes were characterized using flow cytometry, intracellular staining, qRT-PCR, and functional assays. Infections of iPSC-derived hepatocytes were done using a recombinant vesicular stomatitis virus expressing the EBOV surface glycoprotein (VSV-Z76-GFP). Antiviral responses and interferon production were analyzed with qRT-PCR.

Results: Our hepatocytes expressed typical hepatic markers and CY3A4 was shown to be active. These cells were capable of binding LDL and contained LDL-rich vesicles. Once differentiated, our iPSC-derived hepatocytes were susceptible to infection. We also infected iPSCs and differentiated endoderm cells with VSV-Z76-GFP, and they were not susceptible to infection. During infection, our iPSC-derived hepatocytes expressed interferons. Our iPSC-derived hepatocytes expressed surface markers that are involved in EBOV entry and differentially expressed these markers throughout the course of infection.

Discussion: We provide evidence that we are able to robustly generate hepatocytes from human iPSCs. These cells are functionally and transcriptionally similar to mature hepatocytes. Our iPSC-derived hepatocytes are susceptible to viral infection and capable of mounting an antiviral response.

Conclusion: Our iPSC-derived hepatocytes are a suitable in vitro model for Ebola infection in the human liver. We can now use this model to better understand the mechanisms underlying how the human liver supports EBOV replication and pathogenesis.

34. EXOSOMES: A VEHICLE FOR INTERCELLULAR COMMUNICATION AND A NOVEL FOCUS FOR REGENERATIVE DIABETES THERAPIES

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Background: Exosomes are membrane bound vesicles secreted from most cells. Exosomes can transfer protein and genetic factors across the extracellular space, a behavior known to play a role in both healthy and pathogenic intercellular interactions. Stem cells have been found to secrete exosomes carrying proteins and microRNAs with therapeutic properties related to tissue repair and angiogenesis. We hypothesize that the reparative properties of BMSC-derived exosomes can improve the survival and function of human pancreatic islets. Our preliminary results support our hypothesis.

Methods: We isolated bone-marrow derived exosomes and applied them to cultured human islets. After one month of cell culture: media insulin levels and islet beta cell activity were compared between islets cocultured with BM-EXOs, islets cocultured with bone marrow mesenchymal stem cells (BMMSC), and islets cultured alone. Media Insulin levels were evaluated using insulin ELISA assays, and anti-insulin



antibody fluorescent immunohistochemistry was used to observe any change in islet area and β cell population.

Results: We found that Islets cultured with isolated BM-EXOs for one month displayed similar insulin secretion compared to islets cocultured with BMMSCs, and significantly elevated insulin secretion compared to islets cultured in isolation. Fluorescent anti-insulin antibody staining also revealed that islets treated with BM-EXOs had a beta cell population and cross-sectional area similar to islets cocultured with BMMSCs, and a significantly greater cross-sectional area and number of beta cells compared to islets cultured alone.

Discussion: BM-EXOs improved human islet β cell function and increased β cell population in vitro. Due to the similarities in effect between BM-EXOs and BMMSC coculture, BM-EXOs may be a mechanism of the reparative and protective properties of BMSCs in islet coculture.

Conclusion: Our preliminary data supports our hypothesis that BM-EXO is able to support human islet function in vitro. Further study is needed to identify the mechanisms of this effect, and the ability of BMEXOs to support islet survival over longer periods and in vivo.

35. RARE CODING MUTATIONS IN ALZHEIMER DISEASE

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Objective: Much of the unexplained heritability of Alzheimer disease (AD) may be due in part to rare genetic variants whose effects are not captured in most GWAS. Because very large samples are needed to observe statistically significant associations with rare variants in most genome-wide approaches, we applied a strategy that focused on rare variants occurring only in cases or controls.

Methods: Whole-exome sequencing (WES) performed as part of Alzheimer's Disease Sequencing Project (ADSP) on non-Hispanic whites (5617 cases, 4594 controls). The number of minor alleles was tabulated for rare variants occurring in only AD cases or controls in 110 genes previously associated with AD or dementia.

Results: rs149307620 in NOTCH3 had the largest number of rare alleles in AD but none in controls and was previously reported for CADASIL, a diagnostically distinct disorder marked by stroke and dementia. Other interesting mutations only found in at least 4 cases in AD genes include: previously reported AD variants such as rs63749824 in PSEN1 (n=7), rs139710266 in SORL1 (n=5), rs104894002 in TREM2 (n=4), and E1679X in ABCA7 (n=4) with TREM2 and ABCA7 having high impact stop-gain mutations.; an unreported PSEN1 mutation, rs375376095, detected very close to known AD PSEN1 rs63749824; and unreported high impact splice site variant rs376824416 in ABCA7. A novel protective variant in GLIS3, found in controls but not in cases, is located 3010 bp from previously AD associated protective intronic variant, rs514716.

Interpretation: We identified potential risk and protective AD rare variants. Next steps include extending the search genome-wide, performing gene-based tests of aggregated variants, and replication in independent samples. These variants, many of which are predicted to have high impact on protein level or structure, may be useful for future studies aimed at improving our understanding of the basis of AD and developing novel therapeutic targets.



36. USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS TO MODEL ABCA3 DYSFUNCTION IN VITRO

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INTRODUCTION: Childhood interstitial lung disease (chILD) can be caused by autosomal recessive mutations in ATP binding cassette member A3 (ABCA3), a lamellar body associated lipid transporter expressed in alveolar epithelial type II cells (AEC2s). Dysfunction of ABCA3 is thought to cause AEC2 injury by disrupting surfactant biogenesis, resulting in lung remodeling. AEC2s are difficult to study in cell culture due to their propensity to transdifferentiate, and inability to adequately proliferate. Using patient-specific induced pluripotent stem cells (iPSCs) as an inexhaustible source of AEC2s, we sought to engineer an *in vitro* model of ABCA3 deficiency.

METHODS: We reprogrammed blood and fibroblast samples of patients with homozygous E690K, W308R, and 806insGCT ABCA3 mutations spanning two major types of ABCA3 mutations. Using gene editing technology, we targeted a Tomato fluorochrome reporter to the Surfactant protein C (SFTPC) gene, the first locus specifically activated during the differentiation of distal lung epithelial progenitors.

RESULTS: Using this reporter, we established a protocol which generated organoids containing putative Tomato+ cells that expressed AEC2-specific transcripts at levels similar to primary lung tissue controls by RT-qPCR and observed lamellar bodies by electron microscopy. Using this protocol, untargeted E690K iPSC were differentiated to organoids in 3D matrigel at which time they robustly expressed transcripts specific to AEC2s including—ABCA3, LPCAT, and SFTPC. Furthermore, we generated a SFTPC Tomato targeted E690K line which will enable us to identify, purity, and study AEC2-specific effects of ABCA3 deficiency.

DISCUSSION: We demonstrated the ability to generate putative AECs of sufficient maturity to potentially model chILD caused by mutations in ABCA3. Using this protocol, we will generate AEC2 models of different ABCA3 mutations compared to their gene-corrected controls.

CONCLUSION: Upon complete characterization, these models will allow robust testing of novel drugs and gene editing techniques for potential use in patients with ABCA3 mutations.

37. DISTINCT IMMUNE PROFILES IN SURVIVORS VS. NONSURVIVORS OF SEPSIS

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Introduction: Immune dysfunction is a major component of the pathophysiology due to sepsis, however much is still not understood about the mechanisms contributing to this process, which has limited the development of effective therapeutics. Our goal was to examine different mediators of immune signaling to determine mechanistic explanations for this dysfunction, and determine if blockade of PD-L1 would alter survival in sepsis.



Methods: Outbred CD-1 mice underwent cecal ligation and puncture (CLP) to induce sepsis. 24h postCLP, plasma cytokines were measured by ELISA. Splenocytes were analyzed by flow cytometry for expression of MHCII, co signaling molecules (CSM), and markers of activation. Additional mice were treated 6h post CLP with anti PD-L1 and followed for survival.

Results: Mice were stratified into groups predicted to live (P-Live) or die (P-Die) on the basis of plasma IL6. P-Die mice had greater expression of MHCII compared to P-Live mice. All septic mice had increased expression of PD-L1 on splenic monocytes relative to healthy mice, however P-Die mice expressed higher levels relative to P-Live mice. P-Die sustained a greater amount of lymphopenia. Lymphocytes in P-Live mice had higher expression of CD28, CD40L and CD25 (IL-2R), but lower expression of CD69 compared to those from P-Die mice. Surprisingly, P-Live mice that received anti PD L1 had a significantly higher mortality than P-Live mice treated with isotype.

Conclusions: Studies of CSM, MHCII and activation markers in septic mice demonstrate distinct differences in the immunoprofile of survivors and non-survivors. Cells in P-Die mice are capable of presenting antigen, but may induce apoptosis of lymphocytes via increased PD-L1 signaling. Lymphocytes from P-Live mice show markers of greater activity and effector function. These findings improve our understanding of immune dysfunction in the septic host. Immunoprofiling of septic patients will identify survivors vs. non-survivors and guide appropriate immunotherapy of sepsis.

38. IDENTIFICATION OF FOOD-GRADE SUBTILISINS AS GLUTEN-DEGRADING ENZYMES TO TREAT CELIAC DISEASE

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Background: Gluten, the storage protein of wheat, barley and rye, consists of proline and glutamine-rich proteins which contain the immunogenic sequences that drive the destructive intestinal T cell response in celiac disease. *Rothia mucilaginosa*, a harmless oral colonizer, can cleave these gluten epitopes. The aim was to identify the enzymes and evaluate their potential as novel enzyme therapeutics.

Methods: The membrane-associated *R. mucilaginosa* proteins were extracted and separated by DEAE chromatography. Enzyme activities were monitored with paranitroanilide derivatized- and FRET peptide substrates, and by gliadin zymography. Epitope elimination was determined by ELISA. Gliadin-degrading enzymes were identified by LC-ESI-MS/MS.

Results: The *Rothia* proteins with enzymatic activity were identified as hypothetical proteins. A blast search revealed that these are subtilisin-like serine proteases belonging to the peptidase S8 family. Alignment of the major *Rothia* subtilisins indicated that all contain the catalytic triad with Asp (D), His (H) and Ser (S) in the D-H-S order. They cleaved succinyl-Ala-Ala-Pro-Phe-paranitroanilide, a substrate for subtilisin with Pro in the P2 position, as in Tyr-Pro-Gln and Leu-Pro-Tyr, cleavage specificities that we reported earlier for the *Rothia* enzymes. Moreover, FRET substrates of gliadin immunogenic epitopes comprising Xaa-Pro-Xaa motives were rapidly hydrolyzed. The *Rothia* subtilisins as well as another subtilisin from *Bacillus licheniformis*, subtilisin A, could rapidly degrade the highly immunogenic gliadin-derived 33-mer peptide. Other major gluten antigenic epitopes were efficiently eliminated by both subtilisins, as demonstrated with R5 and G12 gliadin ELISA assays.

Discussion: A novel class of gluten-degrading enzymes was isolated from *Rothia* bacteria, which were identified as subtilisins belonging to the S8 family of peptidases. Food-grade *Bacillus* species also produce such subtilisins, and these were also able to cleave and abolish gluten immunogenic epitopes.



Conclusion: This study identified Rothia and food-grade Bacillus subtilis as promising new candidates for enzyme therapeutics in celiac disease.

39. IRISIN DEVELOPS PROTECTIVE EFFECTS IN CARDIOMYOBLASTS EXPOSED TO HYPOXIA/REOXYGENATION

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Background: Irisin is a recently identified myokine that brings increases in energy expenditure and contributes to the beneficial effects of exercise by inducing the browning of white adipose tissues. However, irisin's effects in the heart remains unknown. Histone deacetylases (HDAC) have emerged as important mechanisms in the regulation of a variety of cellular responses. HDAC inhibition's cardioprotective effects against injury are well identified. In this study, we will determine 1) the effects of irisin on hypoxia/reoxygenation-induced injury in cardiomyoblasts; 2) whether these effects are associated with mitochondrial function; 3) whether irisin can rescue the detrimental effects of HDAC4 over-expression in cardiomyocytes.

Hypothesis: This study sought to determine the protective effects of irisin on hypoxia/reoxygenation injury (H/R) and its relationship with HDAC4.

Methods: Wild type and stable HDAC4-overexpression cells were generated from H9c2 cardiomyoblasts. HDAC4 overexpression cells and wild type H9c2 cells were exposed to 24 hours of hypoxia followed by one hour of reoxygenation *in vitro* in the presence or absence of irisin (5 ng/ml). Cell cytotoxicity, apoptosis, mitochondrial respiration, and mitochondrial permeability transition pore (mPTP) were determined.

Results: As compared to wild type H9c2 cells subjected to normoxia, hypoxia treatments resulted in an increase in cell death as evident by the increase in lactate dehydrogenase (LDH) leakage, caspase-3 positive cells, apoptotic mitochondria, and increased mPTP, which were attenuated by irisin treatment. However, the irisin-induced improvement in reduction of cell death and improvement in mitochondrial function were suppressed in HDAC4 overexpression cardiomyoblasts exposed to hypoxia and reoxygenation.

Discussion: These results indicate that irisin produces protective effects against hypoxia/reoxygenation injury, which is dependent upon HDAC4. Our study demonstrated that irisin prevents cell death, increases cell survival, and reduces apoptosis in cardiomyocytes exposed to H/R.

Conclusion: Treatment with irisin rescued cardiomyocytes from the detrimental effects of HDAC4 overexpression under hypoxia/reoxygenation.



