Bacterial Colonization Stimulates a Complex Physiological Response in the Immature Human Intestinal Epithelium. DAVID R. HILL 1, Sha Huang 1, Courtney Lynn 2, Disharee Mukherjee 3, Brooke Bons 2, Shrikar Thodla 1, Priya H. Dedia 4, Alana M. Chin 5, Yu-Hwai Tsai 1, Melinda S. Nagy 1, Thomas Schmidt 3, Seth Walk 6, Vincent B. Young 1, 2, 3, and Jason R. Spence 1, 5. 1Department of Internal Medicine, Division of Gastroenterology, University of Michigan, Ann Arbor MI 48109; 2Department of Internal Medicine, Division of Infectious Disease, University of Michigan, Ann Arbor MI 48109; 3Department of Microbiology and Immunology, University of Michigan, Ann Arbor MI 48109; 4Department of Surgery, University of Michigan, Ann Arbor MI 48109; 5Department of Cell and Developmental Biology, University of Michigan, Ann Arbor MI 48109; and 6Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59717. Email: hilldr@med.umich.edu

Introduction: The human gastrointestinal tract is immature at birth, yet must adapt to dramatic changes such as oral nutrition and microbial colonization. The confluence of these factors can lead to severe inflammatory disease in premature infants; however, investigating complex environment-host interactions is difficult due to limited access to immature human tissue. Objective: The aim of the present study is to evaluate the hypothesis that postnatal bacterial colonization promotes multifaceted intestinal maturation. Methods: We utilized human intestinal organoid (HIO) tissue derived from pluripotent stem cells through directed differentiation. This system recapitulates the features of fetal intestinal tissue in vitro. HIOs were co-cultured with commensal E. coli using microinjection to introduce 10^5 CFU E. coli into the lumen of each HIO. Results: Here, we demonstrate that the epithelium of human pluripotent stem cell-derived human intestinal organoids is globally similar to the immature human epithelium and we utilize HIOs to investigate complex host-microbe interactions in this naïve epithelium. Our findings demonstrate that the immature epithelium is intrinsically capable of establishing a stable host-microbe symbiosis. Microbial colonization leads to complex contact and hypoxia driven responses resulting in increased antimicrobial peptide production, maturation of the mucus layer, and improved barrier function. Conclusion: Co-culture of E. coli within the lumen of HIOs results in stable microbial colonization and the induction of mucus expression, epithelial hypoxia, and enhanced epithelial barrier function. These studies lay the groundwork for an improved mechanistic understanding of how colonization influences development of the immature human intestine.

Understanding Global Molecular Chaperone Assemblies Using Cross-linking Mass Spectrometry. ANDREW TRUMAN. University of North Carolina at Charlotte, 9201 University City Blvd., Woodward Hall 486C, Charlotte, NC 28223. Email: atruman1@uncc.edu

Heat Shock Protein 70 (Hsp70) is an evolutionarily well-conserved molecular chaperone involved in several cellular processes such as folding of proteins, modulating protein-protein interactions and transport of proteins across membranes. Previous proteomic studies have suggested that Hsp70 interacts with a large proportion of the proteins in the cell, it remains unclear how many of these are indirect (bridged) interactions. Hsp70 has several functional domains; the N-terminal ATPase domain, the middle linker domain, C-terminal peptide binding domain and lid domain. While these domains have been well studied, the classes of proteins binding to each Hsp70 domain have not been fully examined. We set out to address these fundamental questions utilizing cross-linking mass spectrometry. Complexes of yeast Hsp70 (Ssa1) were purified by FPLC and cross-linked with a novel MS-cleavable molecule disuccinimidyl sulfoxide (DSSO). The cross-linked complex was digested to peptides and the resulting cross-linked peptides were analyzed in a Thermo Fusion Lumos Trivid Mass spectrometer. Peptide matching was achieved using the XlinkX software. After stringent filtering, 202 cross-linked peptides were characterized, with 79% of these representing direct Ssa1-protein interactions.
Although the C-terminus has been traditionally defined as the region for substrate binding and refolding, many of the direct Hsp70 interactions seen were at the N-terminus (80% of interactors bound to the N-terminus, 10% to the substrate binding domain and 10% to the lid region). Interestingly, 29% of the cross-links observed were Ssa1-Ssa1 peptides. Many of the interlinked Ssa1 peptides came from regions on Hsp70 greater than a distance of 30 Å apart (length of the spacer arm of DSSO) ruling them out as possible intra-Ssa1 interactions. His data taken together provides the first evidence that at least a proportion of Ssa1 exists in a dimeric form. We are currently validating these results with truncation constructs of Hsp70 along with Hsp70 containing point mutations at Hsp70-client interaction sites. In conjunction with this, we are analyzing equivalent Hsp70 complexes from mammalian cells.

A-5

Breathprinting Reveals Malaria-Associated Biomarkers and Mosquito Attractants. A. BERNA1, C. Schaber1,2, N. Katta3, L. B. Bollinger3, M. Mwale4, R. Mlotha-Mitole5,6, I. Trehan1,5,6, B. Raman1, and A. R. Odom John1,2. 1Department of Pediatrics, Washington University School of Medicine, St. Louis, MO 63110; 2Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110; 3Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO 63104. Email: a.berna@wustl.edu

Background: Evidence suggests that the malaria parasite manipulates host odors to augment Anopheles mosquito attraction to infected hosts. In vitro studies have shown that parasite-infected red blood cells release plant-like volatile organic compounds (VOCs) called terpenes, including the monoterpene alpha-pinene, which attract mosquitoes (Kelly et al., mBio, 2016). Here we investigate whether malaria-associated mosquito attractants are enriched during natural human malaria infection in an endemic setting. Methods: We therefore collected and analyzed breath samples from an independent cohort of pediatric patients with and without uncomplicated P. falciparum malaria. In brief, children aged 4–16 who presented at Kamuzu Central Hospital and Bwaila Health Centre in Lilongwe, Malawi were consented and enrolled. We collected breath samples onto sorbent tubes, prior to analysis by thermal desorption gas chromatography-mass spectrometry (TD/GC-MS). Results: The malaria-infected group displayed significantly elevated levels of the known mosquito attractants alpha-pinene and 3-carene. Each of these compounds proved to have high specificity for predicting malaria infection (>94%), and moderate sensitivity (41–53%). In addition, we find that malaria infection causes an overall shift in breath composition and we identify six specific biomarkers that successfully classified infection status with 83% accuracy and that may be targeted for noninvasive malaria diagnosis (Schaber et al., JID, 2018). Conclusions: Together, our data provide robust evidence that infections, such as malaria, lead to specific, reproducible changes in breath VOCs. Our also work provides a specific chemical explanation for the finding that malaria infection increases vector attraction, indicating that the malaria parasite hijacks mosquito behavior to increase transmission. Future studies will examine the reproducibility of these findings in heterogeneous locations and populations.

A-7

MicroRNA-138 Inhibits Osteogenic Differentiation and Mineralization of Human Dedifferentiated Chondrocytes by Regulating RhoC and the Actin Cytoskeleton. A. MCALINDEN1, H. Zheng1, D. Ramnaraign2, B. Anderson3, and R. Nunley1. 1Department of Orthopedic Surgery, Washington University School of Medicine, 660 S. Euclid Avenue, St Louis, MO 63110 and 2Saint Louis University School of Medicine, 1402 S. Grand Blvd, St Louis, MO 63104. Email: mcalindena@wustl.edu

The goal of this study was to determine if dedifferentiated chondrocytes isolated from human osteoarthritic (OA) cartilage could be induced toward the osteogenic lineage and how microRNA-138 (miR-138) affects this process. We selected miR-138 based on our previous microarray study where this miRNA was found to be differentially expressed during human growth plate development in long bones. Human primary articular chondrocytes were harvested from OA knee joints following total knee arthroscopy. Cells were then passaged up to four times to generate primary dedifferentiated chondrocyte (DDC) cell lines. DDCs in monolayer culture were transduced with lentivirus expressing either precursor-miR-138 (LV-138) or a non-silencing control RNA (LV-NS) and cultured with lentivirus expressing either precursor-miR-138 (LV-138) or a non-silencing control RNA (LV-NS) and cultured for up to 14 days in osteogenic medium. We found that LV-138 transduction inhibited osteogenic differentiation as indicated by a significant decrease in expression of key osteogenic markers, RUNX2 and osteocalcin. Extracellular matrix calcification and hydroxyapatite formation was also substantially inhibited by miR-138 over-expression. In addition to differentiation, we also found that miR-138 inhibited DDC proliferation by using the BrdU Cell Proliferation Assay ELISA kit (Abcam). Also, the morphology of LV-138-transduced cells was altered whereby cells became more flattened and spread-out. This suggested that cell polarity may be affected which could indicate abnormalities in cell migration and the actin cytoskeleton. Scratch assays and
immunostaining with Phalloidin-iFluor 488 reagent (Abcam) revealed that miR-138 could inhibit cell migration as well as F-actin polymerization, respectively. Computational approaches were utilized (Targetscan, miRTarBase) to identify miR-138 target genes. The small GTPase, RhoC, was identified as a target and was pursued further based on its reported functions in other systems (i.e. regulation of cell migration and cytoskeleton). Indeed, we found that RhoC protein levels were suppressed in DDCs over-expressing miR-138, suggesting the miR-138 targets the 3'UTR of RhoC resulting in inhibition of translation. A series of rescue experiments were then performed to determine if RhoC over-expression (via lentiviral transduction) could attenuate the inhibitory effects of miR-138. We found that DDC proliferation, migration and F-actin polymerization were partially rescued by RhoC. Importantly, RhoC was also able to significantly enhance DDC osteogenic differentiation not only in 2D cultures but also within more physiologically relevant human 3D demineralized bone scaffolds. Quantification of micro-CT images revealed significantly increased bone tissue volume and mineralization content within scaffolds seeded with cells expressing both miR-138 and RhoC. In conclusion, we have identified a mechanism whereby miR-138, via suppression of RhoC, can inhibit osteogenesis. Future studies will be aimed at attempting to target miR-138 or RhoC to modulate bone formation in animal models in vivo.

A-8

Generation of Human Neurons Through MicroRNA-mediated Direct Conversion of Human Adult Fibroblasts and Disease Modeling. A. S. YOO, M. B. Victor, D. G. Abernathy, W. K. Kim, M. J. McCoy, M. Richner, C. J. Huh, H. E. Olsen, Y. Liu, Y. L. Lu, J. Ho, and S. W. Lee. Department of Developmental Biology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110. Email: yooa@wustl.edu

The ability to generate human neurons in culture through reprogramming easily obtainable somatic cells such as skin fibroblasts will offer valuable tools to investigate the pathogenesis of neurological disorders using patient-specific neurons. Brain-enriched microRNAs (miRNAs), miR-9/9* and miR-124 (miR-9/9*-124) are potent neurogenic effectors that when ectopically expressed, display cell fate reprogramming activities to directly convert primary human fibroblasts to neurons. This reprogramming activity relies on the property of miR-9/9*-124 that simultaneously target the transcripts of anti-neurogenic genes including the subunits of chromatin remodeling complexes and transcription factors that repress the expression of neuronal genes. Through these activities, miR-9/9*-124 was demonstrated to trigger an extensive reconfiguration of the chromatin landscape and transcriptome switch resulting in the erasure of the fibroblast fate and acquisition of neuronal identity. This miRNA-induced neuronal state could then synergize with additional transcription factors enriched in distinct brain regions to generate specific subtypes of neurons including cortical neurons, motor neurons, and striatal neurons. The subtype-specific neuronal conversion then offers the potential of modeling inherited neurological disorders that affect discrete subtypes of neurons with patient-specific neurons. Also, we recently discovered that directly converted neurons retain the age stored in the original fibroblasts, allowing the generation of neurons that reflect the age of fibroblast donors, a feature beneficial for modeling adult-onset neurological disorders. Given these findings, we derive striatal medium spiny neurons (MSNs), a primary cell type affected in Huntington’s disease (HD), through direct neuronal conversion of the fibroblasts of HD patients as a cellular model of HD, and found that HD-MSNs manifested many hallmarks of HD pathology. Our results establish direct neuronal reprogramming as an experimental strategy to model adult-onset neurodegenerative disorders.

A-9

P53 Regulation of Osteoblast Differentiation Is Mediated Through Specific MicroRNAs. NALINI CHANDAR1,2, Oliver Couture1, Teresa Kusper1,3, Mustafa Broachwalla2, Lauren Alt3, Elisha Pendleton1, Michael Fay1, and Shivang Shah1,3. 1Department of Biochemistry, 2Chicago College of Osteopathic Medicine, and 3College of Health Sciences, Midwestern University, Downers Grove, IL 60515. Email: nchand@midwestern.edu

We have previously shown a role for the tumor suppressor gene p53, in transcriptional activation of several bone-specific genes in osteoblast differentiation. In this study, we used a screen for microRNAs to look for ones that were p53 dependent and differentiation dependent. In a model of in vitro osteoblast differentiation, our previous work has established that p53 levels generally increase around day 4 of differentiation and represent the timeline when p53 transcriptionally regulates bone-specific gene expression. In these experiments, we used MC3T3-E1 osteoblasts to stably reduce p53 levels using specific shRNAs. MicroRNAs were isolated from day 0 and day 4 after treatment with differentiation promoting (DP) media. A microRNA profiling service utilizing a microarray detection system analyzed over 1000 different microRNAs in control and DP treated cells to determine differentiation specific and p53 dependent microRNA expression. We validated several of the changes using real-time PCR and selected two microRNAs to study in detail. These two microRNAs (miR34b and 140) were increased two-fold during normal differentiation but showed a dramatic reduction in expression when p53 levels were reduced. We ectopically expressed these
microRNAs in MC3T3-E1 cells and created stable lines. Cells carrying miR34b showed a decrease in cell proliferation rates when compared to miR140 expressing and control cells. P53 levels directly correlated with miR34b expression but not with miR140. MiR140 ectopic expression increased bone morphogenetic protein 2 (BMP2) activity, an important bone anabolic agent that is also p53 regulated. Putative target binding sites for bone-specific transcription factors Runx2, SP7, Vitamin D receptor (VDR) were found for miR34b and 140 and were utilized in luciferase reporter assays to confirm microRNA specific interactions. These studies provided evidence and confirmed for us that p53 regulation of osteoblast differentiation is also mediated through specific microRNAs that directly target important bone-specific genes.

A-11

Pragmatic Use of State-of-the-Art Lung Models to Evaluate Exposure-induced, Adverse Pulmonary Effects. HOLGER P. BEHRSING1, Mario Aragon1, Allison Hilberer1, Devin Sheehan1, and Khalid Amin2. 1Institute for In Vitro Sciences, Inc., Respiratory Toxicology, 30 W. Watkins Mill Rd. Ste. 100, Gaithersburg, MD 20878 and 2University of Minnesota, Laboratory Medicine and Pathology, Minneapolis, MN 55455. Email: hbehrsing@iivs.org

With the expected increase in novel chemicals, a greater demand for cost-effective toxicity testing of new chemicals follows suit. The 2007 report, “Toxicity Testing in the 21st Century – a Vision and a Strategy”, The National Academies Press, Washington D.C., describes a path forward for toxicology and envisions the use of more predictive human-relevant in vitro models for estimating human health risks. Aside from the ethical considerations and often poor predictive value of animal testing, the vast numbers of chemicals requiring evaluation will require a new strategy that many researchers believe depends upon the use of competent human-relevant, in vitro/ex vivo test systems applied in a pragmatic manner. In vitro models utilizing human-derived cell lines and primary cells provide endpoints that reflect cytotoxic, genotoxic, and other relevant adverse events following exposure to toxicants. More recently, the emergence of modern three-dimensional (3D) tissue culture systems of the respiratory tract provide toxicologists with test platforms that more accurately model the complex processes observed in native tissues that involve multiple cell types. Human donor-tissue derived spheroids/organoids, reconstructed human airways, and precision-cut lung slices provide conventional toxicity endpoints as well as more complex, relevant events that follow chemical exposure. The complement of multiple cell types, physiological structure, relevant toxicokinetics, and other properties of these models allow additional evaluations, including chronic-exposure related (e.g. inflammation), functional (e.g. ciliary beating assays), and other endpoints (e.g. Goblet cell hyperplasia) that can reflect more serious health complications that may lead to chronic obstructive pulmonary disease. This presentation will provide an overview of in vitro/ex vivo pulmonary models and how they can be incorporated into a screening/testing strategy for chemicals that can include more complex human pulmonary adverse events that are evaluable in modern 3D systems.

A-12

Prediction of Drug-Induced Gastrointestinal (GI) Toxicity in a 3D Human Small Intestinal Tissue Model. SEYOUM AYEHUNIE. MatTek Corporation, 200 Homer Avenue, Ashland, MA 01721. Email: sayehunie@mattek.com

Gastrointestinal (GI) toxicity cause problems and creates a burden on the health care system and the pharmaceutical industry. Gastrointestinal toxicity often leads to late-stage drug attrition or chronic diseases. In vitro models are needed to guide the design of molecules with lower risk and/or to develop dosing schedules that mitigate the risks in humans. Traditional in vitro cell cultures approach with immortalized human cell lines have been widely applied for assessing drug permeation properties but these cells have unphysiological barrier properties and have not yielded validated assays for GI toxicity. As a result, current preclinical tests for GI safety depend on in vivo testing in higher-order species to achieve translational accuracy. However, animal models also have fundamental problems since they are expensive and do not adequately recapitulate human physiology. In this talk, the functionality and limitations of a novel in vitro 3D primary human small intestine cell-based tissue model for GI toxicity studies will be discussed. The characterization of the reconstructed 3D intestinal tissue model including differentiation and cellular phenotypes, drug transporters and metabolizing enzymes gene expression levels, and biomarkers that can better predict human GI toxicity compared to preclinical animal models will be highlighted. The incorporation of the 3D intestinal tissue model into a multi-organ microphysiological body-on-a-chip platform will be demonstrated. Study results that identify therapeutic compounds with a potential of predicting human GI toxicity outcome by the 3D intestinal tissue model but not with dog and rat toxicity studies will be presented. Overall, the 3D intestinal tissue platform could be a useful pre-clinical and investigational tool for risk assessment of drug induced GI toxicity.

A-13

Genome Editing with Group II Introns, ZFNs, and CRISPR-based Systems. GREGORY DAVIS. MilliporeSigma, St.
CRISPR systems function as an adaptive immune system in bacteria and archaea for defense against invading viruses and selfish genetic elements. In recent years, biochemical analysis of CRISPR ribonucleoproteins (RNPs) has uncovered relatively simple and efficient molecular systems composed of single proteins (such as Cas9) and two RNAs which assist in sequence-specific DNA binding (crRNA and tracrRNA). The RNA-guided nature of these biomolecules and their portability to eukaryotic systems has stimulated wide adoption and new waves of technological development in many applied fields. In this talk, several fundamental applications will be covered including genome editing of bacterial and mammalian cells, implementation of purified CRISPR RNPs in living cells (and *in vitro*), high throughput gene knockout, and new approaches at MilliporeSigma to improve CRISPR activity and specificity by addressing limitations of mammalian chromatin.

The Role of Automation in Engineering Target-specific Disease Models and Reporter Cell Lines. E. PATTERSON. MilliporeSigma, Gene Editing and Novel Modalites, 2909 Laclede Ave, St. Louis, MO 63103. Email: ethan.patterson@sial.com

Gene editing has become a firmly established technology within the discovery sciences arena. With the advent of CRISPR/Cas9 systems, the researcher's ability to find an active nuclease for nearly any region of any genome is now a reality. Even with better nucleases available, those who routinely use gene editing tools to manipulate cell lines encounter other significant challenges that pose a barrier to building the "correct" cell line model. As a well-established partner for custom cell line engineering, we at MilliporeSigma have encountered many of these challenges and have developed and/or implemented a number of methods to circumvent them. A summary of the common pain points of gene editing in cell lines with a focus on where automation can be beneficial in this process will be presented.