19th Annual Surgery Research Conference

Biomedical Research Tower, room 115
Friday, May 16, 2014
Welcome

Welcome to the 19th Annual Department of Surgery Research Conference! This conference is designed to bring students, residents, fellows, faculty and guests together to share and discuss results of research relevant to a variety of surgical disciplines. It is also an opportunity for Department of Surgery (DOS) residents, graduate students and postdoctoral research trainees to develop their scientific communication skills. Each year the Department of Surgery invites a leader in Surgery to visit The Ohio State University and get to know the students and faculty in the department through a variety of activities including participation as a faculty judge at the Annual DOS Research Conference. This year we are delighted to have Dr. Anthony A. Meyer, the Colin G. Thomas, Jr., MD Distinguished Professor and Chair, Department of Surgery, University of North Carolina, as our guest. In addition to the visiting professor, we also ask a prominent research leader at Ohio State to participate in our conference by serving on a panel of faculty judges. Joanna Groden, PhD, Vice Dean for Research in the College of Medicine, has generously agreed to participate.

Over the years the format for the conference has developed into two oral sessions separated by a poster session. The oral and poster presentations are competitively selected based on the quality of the science, impact of the work, and novelty and diversity of the topic. DOS faculty serve as “Faculty Discussants” and comment on the presentation to put the work into context for the audience and stimulate additional discussion. We invite medical students on their 3rd year Surgery Clerkship to become active participants by reviewing the topic relevant to the research abstract and preparing questions or comments for the presenter. Many of the residents who participate in this conference are trainees in the Department of Surgery Master of Medical Science Program which includes structured didactics in Research Design, Biostatistics, Research Ethics, Scientific Communication (including grant writing) and Electives relevant to the area of research.

Ginny L. Bumgardner, MD, PhD
Associate Dean for Research Education
Agenda
Friday, May 16, 2014

Welcome and Introduction of Visiting Professor, 8:00 am
Robert S. D. Higgins, MD, MSHA
Professor and Chairman, Department of Surgery
John H. and Mildren C. Lumley Medical Research Chair
Surgeon-in-Chief, Wexner Medical Center
Director, Comprehensive Transplant Center

Introduction to the Conference
Ginny L. Bumgardner, MD, PhD
Professor of Surgery, Division of Transplantation
Associate Dean for Research Education, OSU College of Medicine
Director, Master of Medical Science Program,

Judges
Anthony A. Meyer MD, PhD, Joanna L. Groden, PhD,
Robert S. D. Higgins, MD, MSHA, and E. Christopher Ellison, MD

Moderator
Session 1 and 2 moderated by Ginny Bumgardner, MD, PhD

Session 1: Oral Presentations, 8:15 to 9:30 a.m.
Utilization of Ex-vivo Perfusion for Lung Transplantation. Shaylyn Bennett, MD
• Faculty Advisors: Bryan Whitson, MD, PhD & Sylvester Black, MD, PhD • Discussant: Ahmet Kilic, MD, PhD .................................................................11

Near Infrared Fluorescent Cholangiography Facilitates Identification of Biliary Anatomy During Laparoscopic Cholecystectomy. Sylvester Osayi, MD
• Faculty Advisor: Vimal Narula, MD • Discussant: John Phay, MD ..................12

Integrin-Linked Kinase Overexpression in the Dysregulated Stroma of Pancreatic Cancer and the Impact on Patient Survival. Lawrence Shirley, MD • Faculty Advisors: Ching-shin Chen, PhD & Mark Bloomston, MD • Discussant: Christopher Ellison, MD .................................................................13

Conditional Deletion of Dicer in Keratinocytes Compromise Skin Barrier Function Post Wounding via Induction of P21\(^{WAF1/CIP1}\). Subhadip Ghatak, PhD
• Faculty Advisors: Sashwati Roy, PhD & Chandan Sen, PhD • Discussant: Jianjie Ma, PhD .................................................................14

Wound-site Macrophages from Chronic Wound Patients Regulates Keratinocyte Signaling. Kasturi G. Barki, MD • Faculty Advisor: Sashwati Roy, PhD • Discussant: Gail Besner, MD .................................................................15

The Effect of Three-dimensional versus Two-dimensional Imaging Displays on Task Performance by Laparoscopy-naive Subjects. Joseph Drosdeck, MD, MS
• Faculty Advisor: David Renton, MD • Discussant: Peter Muscarella, MD .................................................................16
Break and Poster Presentations, 9:30 to 10:15 a.m.
Silver-zinc Coupled Bioelectric Dressing Disrupts Bacterial Biofilm by Targeting Quorum Sensing and Antibiotic Resistance. Jaideep Banerjee, MS • Faculty Advisor: Chandan Sen, PhD .................................................................17

Impact of Cardiac Interventions on Graft and Overall Survival in Abdominal Transplant Recipients. Eliza Beal, MD • Faculty Advisors: Bryan Whitson, MD, PhD & Sylvester Black, MD, PhD .........................18

Model for End Stage Liver Disease Predicts 30-day Mortality in Patients With and Without Liver Disease Undergoing Cardiac Surgery. Shaylyn Bennett, MD • Faculty Advisor: Sylvester Black, MD, PhD ..............19

Intraoperative Scintigraphy Using a Large Field of View Portable Gamma Camera can Decrease Intraoperative Times for Parathyroidectomies: Initial Experience. Robert Plews, MD • Faculty Advisor: John Phay, MD ...............20

Tissue Engineered Intestine (TEI) of Human Origin Grown on a Bioabsorbable Polyglycolic Acid (PGA) Scaffold Infused with HB-EGF. Terrence Rager, MD, MS • Faculty Advisor: Gail Besner, MD .............................................21

Clinical and Ultrasound Sequelae of Non-Visualized Calf Veins on Duplex Ultrasonography for Suspected Deep Vein Thrombosis. David Strosberg, MD • Faculty Advisor: Michael Go, MD, MS ..........................................................22

Session 2: Oral Presentations, 10:15 a.m. to 12:00 p.m.
miR-21 Enhances Melanoma Invasiveness via Inhibition of Tissue Inhibitor of Metalloproteinases 3 Expression: In vivo Effects of miR 21 Inhibitor. Nicholas Latchana, MD • Faculty Advisor: William Carson, MD • Discussant: Raphael Pollock, MD, PhD ..................................................23

Detection of Cytomegalovirus in Bronchoalveolar Lavage Fluid. Sara Mansfield, MD • Faculty Advisor: Robert Baiocchi, MD & Charles Cook, MD • Discussant: Susan Moffatt-Bruce, MD, PhD .............................................24

Role of E2f8 in tumor progression, initiation, and ploidy in hepatocellular carcinoma using a Tamoxifen-inducible Sa-Cre murine model. Justin Huntington, MD • Faculty Advisors: Gustavo Leone, PhD & Carl Schmidt, MD • Discussant: Sylvester Black, MD, PhD ..................................................25

Engulfment of Apoptotic Cells by Macrophages: A Role of MicroRNA-21 in the Resolution of Wound Inflammation. Amitava Das, M.Pharm • Faculty Advisor: Sashwati Roy, PhD • Discussant: Mark Bloomston, MD ..............................26

P21-Activated Kinase as a Therapeutic Target in Papillary Thyroid Cancer. Kara Keplinger, MD • Faculty Advisor: Matthew Ringel, PhD • Discussant: Lisa Yee, MD ...............................................................27

Knockout of microRNA-21 Leads to Increased Inflammatory Response During Wound Healing. Mithun Sinha, PhD • Faculty Advisors: Sashwati Roy, PhD & Chandan Sen, PhD • Discussant: William Carson, MD .........................28
Visiting Professor

Anthony A. Meyer, MD, PhD

Dr. Anthony A. Meyer is the Colin G. Thomas, Jr., MD Distinguished Professor of Surgery and Chair of the Department of Surgery at the University of North Carolina School of Medicine.

Dr. Meyer earned his medical degree and his doctorate in immunology and pathology from the University of Chicago. He completed his surgical residency at the University of California at San Francisco and served as assistant professor of surgery and anesthesiology at UCSF before coming to UNC in 1984. Dr. Meyer has served in many roles in the department, among them chief of General Surgery, director of the Surgery Residency Program, medical director of Critical Care for UNC Hospitals, and director of Burn Research.

Dr. Meyer’s specialties include general surgery, trauma, critical care, burns, surgical oncology and immunology. He is certified by the American Board of Surgery and the American Board of Surgical Critical Care. He is a Fellow of both the American College of Surgeons and the Royal College of Surgeons of England. He has co-authored over 160 peer-reviewed journal articles and contributed to numerous book chapters and abstracts. Dr. Meyer is frequently requested as a visiting professor.

A member of the UNC chapter of Alpha Omega Alpha, Dr. Meyer is nationally known for his participation in many professional organizations, including the American Board of Surgery, the American College of Surgeons, and the American Association for the Surgery of Trauma. Dr. Meyer is committed to academic surgery and the education and mentoring of future surgeons and the leaders of tomorrow. He is also listed on Castle Connolly America’s Top Doctors®.
Invited OSU College of Medicine Faculty Judge

Joanna L. Groden, PhD

Joanna L. Groden, PhD, is a Professor and Vice Chair in the Department of Molecular Virology, Immunology and Medical Genetics, and Vice Dean for Research at The Ohio State University College of Medicine. She is a renowned authority on inherited cancers and former Howard Hughes Medical Institute investigator (1997-2004). She has been a distinguished faculty member of the Molecular Virology, Immunology and Medical Genetics department since 2005. She has served as the College of Medicine’s Associate Dean for Basic Science Research (2007 to 2011) and Associate Dean for Graduate Studies (2011 to 2013). She co-directs the COM Biomedical Sciences Graduate Program and co-directs the OSU Howard Hughes Medical Institute Med Into Grad Scholars Program.

The Groden Laboratory has three primary areas of interest: the study of Bloom’s syndrome, an inherited disorder that decreases chromosome stability and increases susceptibility to all types of cancer; the study of inherited disorders that increase susceptibility to colorectal cancer, especially familial adenomatous polyposis coli; and using the mouse as a model organism to learn more about how human cancers form and how we might diagnose and treat them. One aim of the laboratory is to understand the relationship between chromosome stability and neoplasia through study of the BLM helicase. BLM carries out very basic and important tasks within the cell, such as telomere elongation, resolution of stalled replication forks and responses to DNA damage. A second area of focus is to understand the genes that are altered in the development of colorectal cancer. Through study of the APC tumor suppressor and its functions in regulating the Wnt signaling pathway, the Groden Lab is investigating how downstream gene expression is regulated and how APC contributes to cell cycle regulation and apoptosis. In addition, through the ability to manipulate the mouse genome, new mouse models of cancer are being developed and characterized in the laboratory. These model systems provide access to tumor material and are being used to test new ideas about the genes and their variants that affect susceptibility to cancer, as well as important environmental agents that affect tumor development.

Dr. Groden earned her B.A. in biology from Middlebury College, Vermont, and her Ph.D. in cell biology and genetics from Cornell University of Graduate School of Medical Sciences, New York. She completed a postdoctoral fellowship in the Department of Human Genetics at the University of Utah.
Presenters

Jaideep Banerjee, MS
Graduate Research Associate
**Hometown:** Calcutta, India  
**BS:** Physiology, University of Calcutta, Calcutta, India  
**MS:** Molecular Biology, University of Calcutta, Calcutta, India  
**Research interests:** MicroRNAs in tissue injury and repair

Kasturi Barki, MD
Post Doctoral Researcher
**Hometown:** Bangalore, India  
**MD:** Mahadevappa Rampure Medical College, Gulbarga University, Karnataka State, India  
**Research interests:** Inflammation and wound healing

Eliza Beal, MD
General Surgery Resident
**Hometown:** Ann Arbor, MI  
**BS:** Anthropology-Zoology, University of Michigan, Ann Arbor, MI  
**MD:** The Ohio State University, Columbus, OH  
**Research interests:** Solid organ abdominal transplant, hepatocellular carcinoma

Shaylyn Bennett, MD
General Surgery Resident, Master of Medical Science Program Candidate  
**Hometown:** Cambridge, OH  
**BS:** Mathematics, Ohio University, Athens, OH  
**MD:** University of Toledo, Toledo, OH  
**Research interest:** Cardiothoracic surgery, heart and lung transplantation, extracorporeal life support

Amitava Das, M.Pharm
Graduate Research Associate  
**Hometown:** Kolkata, India  
**B. Pharmacy:** PES College of Pharmacy, Bangalore, India  
**M. Pharmacy:** PES College of Pharmacy, Bangalore, India  
**Research interest:** Wound healing, Inflammation, MicroRNA,
Joseph Drosdeck, MD, MS
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Milwaukee, WI
BA: Psychology, University of Wisconsin - Milwaukee, WI
MD: University of Wisconsin, Madison, WI
Additional training: Master of Medical Science, The Ohio State University
Research Interests: Minimally invasive surgery, surgical education

Subhadip Ghatak, PhD
Post-Doctoral Researcher
Hometown: Kolkata, India
BS: Physiology, University of Calcutta, Calcutta, India
MS: Physiology, University of Calcutta, Calcutta, India
PhD: West Bengal University of Health Science, Kolkata, India
Research interests: MicroRNA in tissue injury and repair

Justin Huntington, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Dayton, Ohio
BA: Zoology, Miami University, Oxford, OH
MD: The Ohio State University, Columbus, OH
Research interest: Hepatocellular carcinoma, foregut malignancies.

Kara Keplinger, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Columbus, OH
BS: Molecular Genetics, The Ohio State University, Columbus, OH
MD: The Ohio State University College of Medicine, Columbus, OH
Research interest: Thyroid cancer, cell signaling

Nicholas Latchana, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Toronto, Canada
BMSc: Biochemistry, University of Western Ontario, Canada
MD: Albany Medical College, Albany NY
Research Interest: Cancer biology
Presenters

Sara Mansfield, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Pomeroy, OH
BS: Biological Sciences and German, Ohio University, Athens, OH
MD: University of Cincinnati, Cincinnati, OH
Research interest: Virology, immunology, global surgery

Sylvester Osayi, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Washington, DC
BS: Biology, University of the District of Columbia, Washington, DC
MD: Medical College of Wisconsin, Milwaukee, WI
Research Interest: Minimally invasive surgery, surgical education

Robert Plews, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: New Port Richey, FL
BS: Microbiology, University of South Florida, Tampa, FL
MD: University of South Florida College of Medicine, Tampa, FL
Research interest: Thyroid cancer with particular focus on tumor biology and metabolism

Terrence Rager, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: New Florence, PA
BS: Life Science, Pennsylvania State University, State College, PA
MD: Pennsylvania State University College of Medicine, Hersey, PA
Research interest: Necrotizing enterocolitis, tissue engineering

Lawrence Shirley, MD
Surgical Oncology Fellow, Master of Medical Science Program Candidate
Hometown: Versailles, KY
BA: English and Molecular Biology, Vanderbilt University, Nashville, TN
MD: University of Kentucky College of Medicine, Lexington, KY
Additional training: General surgery residency, Thomas Jefferson University Hospital, Philadelphia, PA
Research interest: Novel therapeutics for pancreatic cancer and neuroendocrine tumors
Presenters

Mithun Sinha, PhD
Post Doctoral Researcher
Hometown: Kolkata, India
BS: Microbiology, University of Calcutta, India
MS: Biotechnology, University of Calcutta, India
PhD: University of Calcutta, India
Research Interest: Molecular biology of wound healing

David Strosberg, MD
General Surgery Resident
Hometown: Albany, NY
BS: Biological Science, University at Albany, State University of New York, Albany, NY
MD: Upstate Medical University, State University of New York, Syracuse, NY
Research interest: Coagulation and hemostasis
Utilization of Ex-Vivo Perfusion for Lung Transplantation

Shaylyn C. Bennett, MD; Sylvester M. Black, MD, PhD; Robert S.D. Higgins, MD, MSHA; Bryan A. Whitson, MD, PhD

Introduction: There is currently a critical organ shortage for lung transplantation with only 17% of offered organs being transplanted. Of those patients able to receive a transplant, 32% will develop severe, grade 3 primary graft dysfunction (PGD), which has a significant detrimental impact on 5-year and 10-year patient survival. PGD has been attributed to ischemia/reperfusion (I/R) injury. Soluble mediators (i.e., cytokines and microRNAs) have been identified as markers of lung injury. One example of a soluble cytokine that has been identified in lung injury and transplant related dysfunction is interleukin 6. MicroRNA 146a has been found to be a marker of pressure-induced inflammation and a regulator of mechano-transduction. Ex-vivo lung perfusion (EVLP) utilizes components of cardiopulmonary bypass to isolate the lung and evaluate its function over time as a means of assessing suitability for transplant. The theorized mechanisms of benefit are removal of interstitial fluid, washing out of inflammatory mediators, and allowing for alveolar recruitment at low airway pressures. Utilizing EVLP, critical, mechanistic steps in lung injury progression will be identified. Elucidating these steps will enable definition of a robust biologic metric to assess lung function. As a result, we will establish therapeutic approaches to mitigate acute lung injury (ALI) and subsequently recondition and protect the lung. Based on our preliminary data, we hypothesize that Ex-Vivo Lung Perfusion (EVLP) can be utilized to define the mechanisms underlying acute lung injury. Further, we predict the EVLP may be utilized to clinically evaluate and remodel organs via implementation of a complementary cytoprotective perfusion strategy targeting these mechanisms of injury thus expanding the donor pool.

Methods: Lungs will be procured from a donor animal, ventilated via a tracheal cannula, and perfused through a pulmonary artery inlet and left atrial outlet. We have developed a perfusion circuit (Figure 1 & 2). Perfusate is removed from the reservoir via a pump and passes through an oxygenator that is used to deoxygenate the perfusate via a 6% O$_2$, 8% CO$_2$, 86% N$_2$ gas mixture. This allows for the determination of lung contribution to oxygenation via pre and post lung pressure and oxygen monitoring ports. The circuit allows for airway pressure and volume as well as temperature modulation of the perfusate. Over the course of an experiment we are able to ascertain: compliance, P/F ratio, pulmonary vascular resistance, lung weight, and oxygen production. We are able to sample the perfusate in line and will do so at 15-30 min intervals. At the end of a perfusion, the right lobes will be used to assess each of the following: wet-to-dry ratio, histology, protein, and miRNA and mRNA changes respectively. The left lobes will be used for transplant analysis, and the perfusate will be assessed for cytokines. The data collected to assess mechano-transduction effects of ventilation will be corroborated with a cell culture model previously developed. Reactive oxygen species and NO production will be evaluated in the endothelium as well as perfusate. The molecular markers will be correlated with physiologic properties: compliance, P/F ratio, pulmonary vascular resistance, lung weight, and oxygen production. The lung assessment profile obtained while on EVLP will be correlated with post-transplant survival and oxygenation. The planned approach is to refine the rat model with a larger sample size and then advance to a porcine model as one that would be more directly translatable to human transplantation.

Conclusions: A better understanding of tissue injury and organ remodeling as well as the development of an organ-specific ex-vivo perfusate could increase the number of transplantable organs as well as the ultimate success of those organs thereby helping to correct the critical organ shortage and improving graft outcomes.
Near Infrared Fluorescent Cholangiography Facilitates Identification of Biliary Anatomy During Laparoscopic Cholecystectomy

Sylvester N. Osayi, MD; Mark R. Wendling, MD; Joseph M. Drosdeck, MD; Umer I. Chaudhry, MD; Kyle A. Perry, MD; Sabrena F. Noria, MD, PhD; Dean J. Mikami, MD; Bradley J. Needleman, MD; Peter Muscarella II, MD; Mahmoud Abdel-Rasoul, MS, MPH; W. Scott Melvin, MD; Jeffrey W. Hazey, MD; Vimal K. Narula, MD.

Introduction: Intraoperative cholangiography (IOC) is the current gold standard for biliary imaging during laparoscopic cholecystectomy (LC). However, utilization of IOC remains low. Near Infrared Fluorescence Cholangiography (NIRF-C) is a novel, non-invasive method for real-time, intraoperative biliary mapping. Our aims were to assess the safety and efficacy of NIRF-C for identification of biliary anatomy during LC.

Methods: Patients were administered indocyanine green (ICG) prior to surgery. NIRF-C was used to identify extrahepatic biliary structures before, and after partial and complete dissection of Calot’s triangle. Routine IOC was performed in each case. Identification of biliary structures using NIRF-C and IOC, and time required to complete each procedure were collected. NIRF-C and IOC procedure costs were estimated.

Results: Eighty-two patients underwent elective LC with NIRF-C and IOC. Mean age and BMI were 42.6±13.7 years and 31.5±8.2 kg/m², respectively. ICG was administered 73.8±26.4 minutes prior to incision. NIRF-C was significantly faster than IOC (1.9±1.7 vs. 11.8±5.3 minutes, p<0.001). IOC was unobtainable in 20 (24.4%) patients while NIRF-C did not visualize biliary structures in 4 (4.9%) patients. After complete dissection, the rates of visualization of the cystic duct, common bile duct, and common hepatic duct using NIRF-C were 95.1%, 76.8%, and 69.5%, respectively, compared to 72.0%, 75.6%, and 74.3% for IOC. In 20 patients where IOC could not be obtained, NIRF-C successfully identified biliary structures in 80% of the cases. Higher BMI was not a deterrent to visualization of anatomy with NIRF-C. No adverse events were observed with NIRF-C. Lastly, NIRF-C was significantly cheaper than IOC ($99 vs. $2,755).

Conclusions: NIRF-C is a safe and effective alternative to IOC for imaging extrahepatic biliary structures during LC. Compared to IOC, utilization of this novel technique could potentially decrease bile duct injury rates at a much lower cost and significantly shorter procedure time.
Integrin-Linked Kinase Overexpression in the Dysregulated Stroma of Pancreatic Cancer and the Impact on Patient Survival

Lawrence A. Shirley, MD; Ming-Chen Yang, PhD; Benjamin Swanson, MD, PhD; Thomas Mace, PhD; Gregory Lesinski, PhD; Wendy Frankel, MD; Tanios Bekaii-Saab, MD; Mark Bloomston, MD; Ching-Shih Chen, PhD

Introduction: Integrin-linked kinase (ILK), a serine/threonine protein kinase that normally plays a role in cell-extracellular matrix interactions, has been shown to promote invasion in pancreatic cancer. Due to these functions of ILK, we wanted to examine ILK expression in the stroma of several pancreatic specimen types and, in cancer, determine if a relationship exists between ILK expression and survival. Additionally, we wanted to examine the role of ILK in one of the primary cell types in dysregulated stroma, activated pancreatic stellate cells.

Methods: A tissue microarray (TMA) of pancreatic cancer samples (n=37) was stained for ILK expression by immunohistochemistry and scored from zero (no expression) to three (high expression) in both tumor and stroma. Stromal ILK expression was also scored in 34 samples of chronic pancreatitis, 28 of intraductal pancreatic mucinous neoplasm (IPMN), and 15 of normal pancreatic tissue. A second TMA of 150 samples was stained for ILK and a clinical database was queried to compare survival based on ILK expression in the tumor and surrounding stroma. Primary activated pancreatic stellate cells (PSCs) were grown from patient samples. These cells were lysed and ILK expression was analyzed and compared to both normal pancreatic epithelial and cancer cell expression by immunoblotting. Transfection of PSCs with lentiviral vector containing shRNA to ILK was performed and cells were examined for morphologic changes and altered protein expression. Paraffin-embedded pancreatic samples from transgenic mice with pancreas-specific mutations in KRAS and p53 (KPC), KPC plus BRCA1, KPC plus BRCA2, as well as normal control mice were stained for ILK expression.

Results: ILK expression was significantly higher in the stroma of pancreatic cancers versus tumors in the same tissue (mean score 2.34 vs. 1.32, P <0.001). Stromal ILK expression was significantly higher in cancer as compared to normal pancreas (mean score 0.73, P<0.001), chronic pancreatitis (mean score 1.38, P<0.001), and IPMN (mean score 1.93, P=0.05). In the TMA of 150 tumor samples, stromal expression, but not tumor expression, was associated with overall survival. Patients with low to no stromal ILK expression (Grades 0 and 1) had a median survival of 21.2 months vs. 13.2 months in Grades 2 and 3 (P=0.016). In activated PSCs, ILK was overexpressed, with increased expression when compared to all pancreatic cancer cell types examined (AsPC-1, MiaPaCa-2, PANC1, SW 1990) in addition to normal pancreatic cells. Immunohistochemical staining of murine samples revealed minimal ILK expression in normal control mice, moderate to high ILK expression in the stroma of KPC and KPC-BRCA2 tumors, and high expression in both the tumor and stroma of the KPC-BRCA1 tumors.

Conclusions: Stromal ILK expression increases from normal pancreas to chronic pancreatitis to IPMN to pancreatic cancer. In patients with cancer, increased expression in the stroma was associated with worse survival, revealing a possible role of ILK in the crosstalk between tumor and stroma and progression of disease. Activated pancreatic stellate cells within the stroma have increased ILK expression. Thus, ILK inhibition may provide a way to specifically target the dysregulated tumor microenvironment while sparing normal stroma. Transgenic mouse models express ILK in the stroma in a similar manner to human samples, providing a viable pre-clinical model to examine pharmacologic ILK inhibition.
Conditional Deletions of Dicer in Keratinocytes Compromise Skin Barrier Function Post Wounding via Induction of P21\textsuperscript{WAF1/CIP1}

Subhadip Ghatak, PhD; Yuk Cheung Chan, PhD; Jaideep Banerjee, MS; Savita Khanna, PhD; Sashwati Roy, PhD; Chandan K. Sen, PhD

**Introduction:** Adult tissue homeostasis is maintained by molecular silencers called microRNAs that post-transcriptionally silence coding genes relevant to tissue growth and repair. Injury transiently silences these silencers to unleash tissue development enabling healing. Once the wound is healed, miRNA biogenesis is bolstered to switch-off tissue development averting neoplasia. We report that Dicer, one of the key RNase III responsible for miRNAs maturation, plays an important role in re-establishing the skin barrier function post wounding. Compromised Dicer function predicts poor health outcomes. Dicer expression is dysregulated in several human disease conditions.

**Methods:** Murine excisional wound model was induced by \(8 \times 16\) mm full-thickness excision on the dorsal skin, equidistant from the midline and adjacent to the four limbs. Expression of miRNA was performed by Luminex microarray. Expression of mRNAs and proteins were validated by quantitative real time PCR and Western blot respectively. Localization of proteins of interest was validated by immunohistochemistry.

**Results:** We observed that non-healing diabetic wounds feature compromised dicer expression. MicroRNA expression profiling of skin and wound-edge tissue revealed global up-regulation of miRNAs during wound closure on day 14 post-wounding during which time Dicer expression was also significantly induced. During wound closure, Dicer protein expression increased by \(>2.5\) fold \((n=4; p<0.001)\). Barrier function of the skin as measured by transepidermal water loss (TEWL) was compromised in keratinocyte-specific Dicer ablated mice because of impaired loricrin expression. In vitro studies with Immortalized human keratinocytes (HaCaT) showed that loricrin expression was inversely related to the expression of the cyclin dependent kinase inhibitor p21\textsuperscript{WAF1/CIP1}. Real time PCR of p21\textsuperscript{waf1/CIP1} from laser captured wound-edge keratinocytes revealed more than \(2.5\) fold elevated mRNA expression in Dicer ablated skin epidermis compared to control epidermis \((n=6; p<0.001)\). Increased expression of p21\textsuperscript{waf1/CIP1} in keratinocyte-specific Dicer ablated wound edge tissue was also confirmed by Western blot and immunohistochemistry. Suppressing p21\textsuperscript{waf1/CIP1} by p21\textsuperscript{waf1/CIP1} anti-sense adenovirus in keratinocyte-specific conditional dicer ablated mice restore the skin barrier function in day 10 post wounding suggesting a role of Dicer in the suppression of p21\textsuperscript{waf1/CIP1}.

**Conclusions:** These results establish that Dicer enables p21\textsuperscript{waf1/CIP1} silencing helping re-establish barrier function of the wounded skin.
Wound Site Macrophages from Chronic Wound Patients Regulates Keratinocyte Signaling

Kasturi Ganesh Barki, MD; Amitava Das, MS; Savita Khanna, PhD; Piya Das, MS; Urmila Gnyawali, RN; Gayle M. Gordillo, MD; Chandan K. Sen, PhD; Sashwati Roy, PhD

**Introduction:** We have successfully isolated functional macrophages from the wound site of chronic wound patients. A transcriptome screening study was performed comparing wound site macrophages with matching blood derived macrophages from the same chronic wound patient. Higher oncostatin M (OSM) expression in wound site macrophages represents a foundation observation on which this study rests. High levels of OSM (p<0.01; n=19) were observed in chronic wound fluids as compared to plasma from the same subjects. OSM is a potent activator of keratinocytes. We hypothesized that OSM produced by wound macrophages interacts with keratinocytes at the wound site to drive cell signaling towards healing.

**Methods:** Adult chronic wound patients (25-80 years old) undergoing VAC® (negative pressure) therapies (NPWT) of their wounds at the Comprehensive Wound Center (CWC) were recruited. Wound fluid was derived from the NPWT dressing by lavaging the wound dressing with saline solution. In addition, paired peripheral blood samples were collected from each patient. Blood monocytes from corresponding subjects with chronic wounds were isolated using a Ficoll-Hypaque density gradient. Human keratinocyte HaCaT were treated either with wound fluid or recombinant OSM.

**Results:** Treatment of human keratinocytes with OSM resulted in dose dependent induction of miRNA-203. miR-203 is abundantly expressed in the skin and is involved with the differentiation of keratinocytes. OSM down regulated keratinocyte SOCS3 protein expression. Elevated SOCS3 impairs epithelial repair of cutaneous wounds by blocking keratinocyte proliferation and migration. The role of SOCS3 in chronic wounds is therefore of outstanding interest. AntagomiR and miR mimic experiments demonstrated that miR-203 directly silences SOCS3.

**Conclusion:** SOCS3 is a key regulatory molecule that inhibits JAK-Stat signaling pathway in keratinocytes. SOCS3 is overexpressed in the wound margin epithelia of diabetic wounds. This work provides first evidence that OSM, produced by human wound-site macrophages, induces miR-203 expression in human keratinocytes silencing SOCS3 and supporting healing.
The Effect of Three-dimensional versus Two-dimensional Imaging Displays on Task Performance by Laparoscopy-naïve Subjects

Joseph M. Drosdeck, MD and David B. Renton, MD

Introduction: Inanimate laparoscopic training techniques such as the Fundamentals of Laparoscopic Surgery (FLS) have a proven benefit on operative skills in trainees with little to no laparoscopic experience. In conjunction with these training techniques, three-dimensional (3D) imaging provides an added benefit to novice trainees with laparoscopic experience. However, the utility of 3D imaging with FLS based tasks in laparoscopy-naïve trainees is unclear. We aim to investigate the role of 3D imaging in laparoscopy-naïve participants using two FLS based tasks. We hypothesize that participants who utilize 3D imaging during these tasks will perform better than participants who utilize two-dimensional (2D) imaging.

Methods: First through fourth year medical students at The Ohio State University were recruited. Participants were randomized to either 3D or 2D imaging prior to performing two FLS tasks – peg transfer and circle cut. For each task, time to completion and number of errors were recorded. A numerical global performance score was calculated for each participant on each task, where higher scores indicate better performance. Scores accounted for time to completion and number of errors committed. A non-parametric test, Wilcoxon Rank Sum Test, was used to draw inferences between experimental groups on peg transfer time, peg transfer score, circle cut time, and circle cut score. Fisher’s Exact Test was employed to draw inferences between experimental groups on peg transfer errors and circle cut errors.

Results: Seventy-nine medical students participated; 39 students were randomized to the 3D group and 40 were randomized to the 2D group. The median peg transfer time was significantly shorter in the 2D group (270 seconds vs. 325 seconds), p = 0.033, and the median peg transfer score was significantly higher in the 2D group (330 vs. -25), p = 0.032. Peg transfer errors did not differ significantly between groups (p = 0.41). Circle cut time, errors, and score did not differ significantly between groups, p = 0.43, 0.65, 0.91, respectively.

Conclusions: Laparoscopy-naïve participants in the 3D group performed similarly to participants in the 2D group on the circle cut task and worse than participants in the 2D group on the peg transfer task. These findings suggest that educators may wish to avoid 3D imaging in laparoscopy-naïve trainees during inanimate laparoscopic training tasks.
Silver-zinc Coupled Bioelectric Dressing Disrupts Bacterial Biofilm by Targeting Quorum Sensing and Antibiotic Resistance

Jaideep Banerjee, MS; Piya Das Ghatak, MS; Savita Khanna, PhD; Sashwati Roy, PhD; Chandan K Sen, PhD

**Introduction:** *Pseudomonas aeruginosa* biofilm is often associated with chronic wound infection. Inspired by clinical case reports we sought to test the anti-biofilm properties of a FDA approved novel bioelectric dressing (BED) that is currently used at our wound center.

**Methods and Results:** BED consists of a matrix of silver-zinc coupled biocompatible microcells, which in the presence of conductive wound exudate gets activated to generate electric field (0.3-0.9V). Growth (O.D and cfu) of pathogenic *Pseudomonas aeruginosa* strain PAO1 in LB media was markedly arrested in the presence of the BED (p<0.05, n=4). PAO1 biofilm was developed in vitro using a polycarbonate filter model. Grown overnight in LB medium at 37°C bacteria were cultured on sterile polycarbonate membrane filters placed on LB agar plates and allowed to form a mature biofilm for 48h. The biofilm was then exposed to BED or placebo for the following 24h. Structural characterization using scanning electron microscopy demonstrated that BED markedly disrupted biofilm integrity in a setting where no significant effect was observed using a commercial silver dressing commonly used for wound care. Staining of extracellular polymeric substance and a vital stain demonstrated decrease in biofilm thickness and number of live bacterial cells in the presence of BED (n=4). BED repressed the expression of quorum sensing genes such as lasI (p<0.05, n=3). BED also down-regulated the activity of glycerol-3-phosphate dehydrogenase, an electric field sensitive enzyme responsible for bacterial respiration, glycolysis, and phospholipid biosynthesis (p<0.05, n=3). Biofilms are antibiotic resistant. Such resistance to aminoglycosides is mediated by elevated tolA and attenuated cytochrome c oxidase III. Both of these critical drivers of antibiotic resistance were sensitive to BED accounting for its ability to fight antibiotic resistance of PAO1.

**Conclusions:** This work presents first evidence on the molecular basis of the anti-biofilm properties of BED.
Impact of Cardiac Interventions on Graft and Overall Survival in Abdominal Transplant Recipients

Eliza W. Beal, MD; Shaylyn C. Bennett, MD; Nikhil P. Jaik, MD; Gary S. Phillips, MAS; Sylvester M. Black, MD, PhD; Todd Pesavento, MD; Robert S.D. Higgins, MD; MSHA; Bryan A. Whitson, MD, PhD

Introduction: Solid organ transplant recipients have a propensity for both having pre-existing and developing cardiovascular disease. End-stage organ dysfunction and immunosuppression may hasten the development. Due to the nature of transplant recipients, interventions are high risk in this population and can affect graft function. We sought to evaluate the impact of cardiovascular interventions (CI) long-term outcomes in abdominal transplant recipients.

Methods: We retrospectively queried a prospectively maintained solid organ transplant database to identify adult recipients undergoing initial transplant over an 11 year period (kidney, kidney-pancreas, or liver) whose continuing-care was performed at our quaternary medical center. We stratified cohorts into CI (percutaneous, coronary artery bypass, valve surgery and complex procedures) and No-CI. We evaluated graft and overall survival. Standard Kaplan-Meier survival analysis, Cox proportional hazard modeling were performed.

Results: During the study period, 714 abdominal organ transplants met study criteria: 140 patients underwent CI and 574 did not. There were no demographic differences. Mean time from transplant to CI was 1360 days. Those patients undergoing renal transplant and CI had a longer graft survival than those undergoing renal transplant no-CI (p=0.013). Late long-term survival (7-14 years) showed a 167% increased risk of death in the CI cohort as compared to no-CI patients in the adjusted Cox proportional hazard model (p=0.003).

Conclusions: While those patients undergoing CI have a longer graft survival and better short-term survival, their long-term survival is significantly decreased compared to those not undergoing CI.
Model for End Stage Liver Disease Predicts 30-Day Mortality in Patients With and Without Liver Disease Undergoing Cardiac Surgery

Shaylyn C. Bennett, MD; Eliza W. Beal, MD; Bryan A. Whitson, MD, PhD; Ravi S. Tripathi, MD; Sylvester M. Black, MD, PhD; John H. Sirak, MD; Chittoor B. Sai-Sudhakar, MBBS; Juan A. Crestanello, MD; Robert S.D. Higgins, MD, MSHA

Introduction: The Model for End-Stage Liver Disease (MELD) score has been developed to predict short term survival in patients with end-stage liver disease. A MELD score of 15 or greater is an indication for consideration for liver transplantation. Its translation to patients undergoing cardiac surgery and the applicability to those without end-stage liver disease is unknown. We sought to determine the utility of MELD score to predict 30-day mortality in a general cardiac surgery population.

Methods: Retrospective analysis of a prospectively maintained outcome database of all patients undergoing cardiac surgery from 7/2011 to 4/2013. The MELD score was calculated by standard formula. Receiver Operator Characteristic (ROC) curve analysis determined MELD values for optimum sensitivity and specificity. The effect of MELD based on the ROC analysis was used to evaluate the impact of a MELD threshold on post-cardiac surgery mortality and outcomes.

Results: There were 1399 patients in the study cohort (437 CABG, 226 isolated valve, 89 CABG+valve). In the overall cohort, a MELD of 10.2 (area under curve (AUC) 0.74, sensitivity (Sn) 0.68, specificity (Sp) 0.62) correlated with 30-day mortality. In those without liver disease, the predictive MELD was 9.1 (AUC 0.73, Sn 0.8, Sp 0.63) and in those with liver disease 14.8 (AUC 0.84, Sn 0.83, Sp 0.37). Stratifying by those with MELD <15, a MELD >=15 was predictive of 30-day mortality and (14.6% v. 3.3%, p<0.0001) overall complications (63.4% v. 37.9, p<0.0001), renal failure (25% v. 5.8%, p<0.0001), dialysis (14% v. 1.3%, p<0.0001), and need for blood products (78.5% v. 37.6%, p<0.0001).

Conclusions: In a general population of cardiac surgery patients, the MELD score is predictive of 30-day mortality though the sensitivity is not as great as in those with liver disease. A MELD of 15 may be a useful predictor of outcomes in those extremely high risk surgical patients.
Intraoperative Scintigraphy Using a Large Field of View Portable Gamma Camera can Decrease Intraoperative Times for Parathyroidectomies: Initial Experience

Robert L. Plews, MD, Nathan C. Hall, MD, PhD, Amit Agrawal, MD, John Phay, MD, Stephen P. Povoski, MD, Chadwick L. Wright, MD, PhD

Introduction: Minimally invasive parathyroidectomy relies on pre-operative localization of a parathyroid adenoma, and intraoperative confirmation of its removal. Adjuncts to intraoperative localization include technetium-sestamibi hand-held probe, confirmation by frozen pathology examination and PTH monitoring. Each modality has its limitation. We investigated a novel technique using intraoperative sestamibi imaging to localize and confirm complete removal of hyperfunctioning parathyroid tissue.

Methods: A portable, large field-of-view gamma camera was used in 20 parathyroidectomy cases for primary hyperparathyroidism. Patients underwent preoperative Tc-99m sulfur colloid injection followed by pre- and post-resection imaging of the neck and resected tissue. Intraoperative PTH was collected at 5 and 10 minute intervals. All specimens were sent for frozen examination.

Results: In all 20 cases, Tc-99m-avid parathyroid glands were visible preoperatively. Corresponding radioactivity in the neck was absent in 13/20 cases following initial resection, indicating successful removal, which was verified by PTH monitoring and pathology. In 7/20 cases residual radioactivity was noted in the thyroid bed or lack of radioactivity on specimen imaging, leading to additional resections until no radioactivity was detectable. Imaging verified complete resection of hyperfunctioning parathyroid tissue in all patients. The mean ± st dev time savings using imaging data to confirm resection versus the first laboratory confirmation result (PTH or Path) was 29.9±8.6 minutes (specimen imaging) and 23.4±7.5 minutes (neck imaging). All patients had biochemical cure demonstrated by postoperative serum PTH and calcium normalization.

Conclusions: Intraoperative nuclear imaging is an effective adjunct for localization and confirmation of parathyroid gland removal in patients with primary hyperparathyroidism. Intraoperative sestamibi imaging can 1) reduce operating time, thereby lowering cost, 2) combined with PTH and pathology, improves accuracy for confirming complete resection.
Tissue Engineered Intestine (TEI) of Human Origin Grown on a Bioabsorbable Polyglycolic Acid (PGA) Scaffold Infused with HB-EGF

Terrence M. Rager, MD, MS; Yanchun Liu, MD; John J. Lannutti, PhD; Palak Painter, MS; Tyler Nelson, PhD; Gail E. Besner, MD.

Introduction: Short bowel syndrome (SBS) typically results from the loss of a clinically significant length of small intestine, and is associated with significant morbidity and mortality. The use of tissue engineered small intestine as a treatment for short bowel syndrome is an attractive alternative to long term total parenteral nutrition (TPN), small bowel transplant, or surgical lengthening procedures. Heparin-binding EGF-like growth factor (HB-EGF) is known to have potent intestinal cytoprotective effects and may benefit the growth and differentiation of implanted intestinal crypt cells during the formation of tissue engineered intestine (TEI). We show results that indicate successful growth of human tissue engineered intestine using human small intestinal crypt cells seeded onto a PGA bioabsorbable scaffold and incubated in the peritoneal cavity of a NOD-SCID mouse.

Methods: A small portion of human small intestinal mucosa (2 cm x 1 cm), procured from a 14 year old undergoing ileostomy takedown was enzymatically digested and filtered to isolate intestinal crypts, and the crypts were suspended in cell culture medium. The suspended crypts were then seeded onto a tubular PGA scaffold which had been infused with HB-EGF under high pressure (900 PSI) for 1 hour in the presence of CO₂. The seeded scaffold was then implanted into the peritoneal cavity of a NOD-SCID mouse for four weeks of in vivo incubation. The scaffold was then explanted, fixed with formalin, paraffin-embedded, sectioned, and mounted for tissue analysis by fluorescence immunohistochemistry (IHC) using an antibody to human β2-microglobulin which specifically recognizes MHC class I molecules on the surface of human nucleated cells only.

Results: Immunohistochemical analysis using a primary antibody specific for human β2-microglobulin and DAPI for nuclear staining revealed a similar structure of our human TEI compared with human native small intestine, which served as positive control (figure 1). Strong staining of human β2-microglobulin was seen in both the TEI sample as well as the human native small intestine positive control. There was absent human β2-microglobulin immunostaining in mouse small intestine (negative control), and there was absent human β2-microglobulin staining in human TEI subjected to the same IHC protocol but without primary antibody to human β2-microglobulin.

Conclusion: Human tissue engineered intestine can be produced from donor human intestinal crypts, seeded onto a bioabsorbable PGA scaffold infused with HB-EGF. Optimization of this technique may hold a future therapy for short bowel syndrome.
Clinical and Ultrasound Sequelae of Non-Visualized Calf Veins on Duplex Ultrasonography for Suspected Deep Vein Thrombosis

Jon C. Henry, MD, MS; David Strosberg, MD; Shantanu Warhadpande, BS; Bhagwan Satiani, MD, MBA; Michael R. Go, MD, MS

Introduction: Calf veins are not visualized in up to 35% of lower extremity venous duplex ultrasounds (DUS). Little is known about the clinical implications of non-visualized calf veins. We sought to investigate the incidence of non-visualized calf veins, rate of subsequent venous thromboembolism (VTE), and factors influencing successful visualization on subsequent DUS.

Methods: We reviewed all patients who had DUS in 2012 at our institution with non-visualized calf veins, no deep vein thrombosis (DVT), and available follow up. Demographics, Well’s score, BMI, reason for DUS, activity level, reason for non-visualization, initial and subsequent DUS results, and subsequent occurrence of VTE were collected.

Results: 8,237 DUS were performed in 2012. 891 (10.8%) DUS in 717 patients had non-visualized calf veins. 484 of these patients had no DVT and had available follow up and comprised the study population. The most common reasons for non-visualization were edema (35.5%) and body habitus (31.8%). 10 pulmonary emboli (PE) and 13 DVT comprising 23 (4.8%) VTE were subsequently identified in the population. No tested variables correlated with development of VTE. 148 of the 484 patients had subsequent DUS at a median of 2.43 months; 45.3% of subsequent DUS successfully imaged the previously non-visualized veins. Lower body weight, whole leg swelling, single vein non-visualization, and single limb non-visualization at initial DUS were associated with successful visualization on subsequent DUS. 13 (8.8%) new DVT were seen on subsequent DUS; 6 were seen in calf veins that previously were not visualized and 7 were seen in either femoral veins or calf veins that previously were visualized and did not have clot.

Conclusions: Non-visualized calf veins are common in DUS. Almost half of patients with non-visualized veins on initial DUS had successful visualization on subsequent DUS. Lower body weight, whole leg swelling, single vein non-visualization, and single limb non-visualization on initial DUS were associated with successful visualization on subsequent DUS. 4.8% of patients with non-visualized veins on initial DUS go on to develop VTE, and 8.8% of patients who have subsequent DUS are found to have DVT. When initial DUS is unable to visualize calf veins, repeat DUS may be useful to identify either new or initially unseen DVT.
miR-21 Enhances Melanoma Invasiveness via Inhibition of Tissue Inhibitor of Metalloproteinases 3 Expression: In vivo Effects of miR 21 Inhibitor

Nicholas Latchana, MD; Sara E. Martin del Campo, MD, MS; Valerie P. Grignol, MD; Kala M. Levine; Ene T. Fairchild, MD, PhD; Alena Cristina Jaime-Ramirez, PhD; Thao-Vi Dao, Volodymyr I. Karpa, Mary Carson, Anthony N. Chan, William E. Carson III, MD

Introduction: Metastatic melanoma is the most aggressive form of this cancer. It is important to understand factors that increase or decrease metastatic activity in order to more effectively research and implement treatments for melanoma. Increased cell invasion through the extracellular matrix is required for metastasis and is enhanced by matrix metalloproteinases (MMPs). Tissue inhibitor of metalloproteinases 3 (TIMP3) inhibits MMP activity. It was previously shown by our group that miR 21, a potential regulator of TIMP3, is over-expressed in cutaneous melanoma. It was therefore hypothesized that increased levels of miR 21 expression would lead to decreased expression of TIMP3 and thereby enhance the invasiveness of melanoma cells.

Methods: WM1552c, WM793b, A375 and MEL 39 metastatic melanoma cell lines were transfected with control miR, pre-miR-21, or anti-TIMP3. Successful transfection was confirmed by Real-Time PCR. Transfected cells were used for invasion, proliferation, and migration assays. Immunoblot analysis was performed on transfected cells for targets of miR 21 including programmed cell death protein (PDCD4), tropomyosin-1 (TM1), phosphatase and tensin homolog (PTEN) with B-actin as an internal control. A375 cells were transfected with oligonucleotides against miR-21 or a control oligonucleotide before injection into the flanks of 01B74 Athymic NCr-nu/nu mice. Tumor growth was analyzed over three weeks. Another group of mice were injected with untransfected A375 cells in the flank and monitored until tumor growth reached an average of 100mm² before injections with PBS alone (control) or anti-miR 21 every three days for four cycles. Tumor growth was monitored and tumor specimens were analyzed for TIMP3 expression by immunohistochemistry using a goat anti-TIMP3.

Results: Immunoblot analysis of miR 21-overexpressing cell lines revealed reduced expression of TIMP3 as compared to controls. This in turn led to a significant increase in the invasiveness of the radial growth phase cell line WM1552c and the vertical growth phase cell line WM793b (p < 0.05), but not in the metastatic cell lines A375 or MEL 39. The proliferation and migration of mir-21 over-expressing cell lines was not affected. Reduced expression of TIMP3 was achieved by siRNA knockdown and significantly enhanced invasion of melanoma cell lines, mimicking the effects of miR-21 over-expression. Treatment of tumor cells with a linked nucleic acid antagonor to miR-21 inhibited tumor growth and increased tumor expression of TIMP3 in vivo. Intra-tumoral injections of anti-miR-21 produced similar effects.

Conclusions: Increased expression of miR-21 enhanced the invasive potential of melanoma cell lines through TIMP3 inhibition. Therefore, inhibition of miR 21 in melanoma may reduce melanoma invasiveness.
Detection of Cytomegalovirus in Bronchoalveolar Lavage Fluid

Sara A Mansfield, MD; Varun Dwivedi, PhD; Joanne Trgovcich, PhD; Charles Cook, MD

Introduction: Cytomegalovirus (CMV) is a virtually ubiquitous pathogen, infecting up to 50-60% of Americans by school age. In healthy individuals, CMV infection is asymptomatic, but goes on to establish lifelong latency. Accumulating data suggest that ~35% of immunocompetent humans have pulmonary reactivation of CMV during critical illness, and that these reactivation episodes are associated with nearly doubled durations of mechanical ventilation, days in the ICU, and mortality. As a diagnostic test of reactivation, some investigators have evaluated bronchoalveolar lavage (BAL) samples from patients for CMV DNA. They postulate that presence of DNA in BAL fluid is indicative of active virus, but data supporting this assumption are lacking. We hypothesized that BAL fluid should be negative for CMV DNA during latency.

Methods: Seventeen Balb/c mice infected with 102 or 106 pfu Smith CMV during latency (1 year after infection) were euthanized. The trachea was cannulized with a 22G angiocatheter. The lungs were lavaged with 3 ml of sterile phosphate buffered saline and BAL samples were collected. DNA was isolated and PCR was performed. Two primer sets were used specific for mCMV: GB1 and GB2.

Results: All 17 mice demonstrated presence of CMV DNA in BALF at low levels (relative expression levels <0.03). Two sets of primers to CMV glycoprotein gB were compared during this analysis (gB1 & gB2). GB1 had an amplification efficiency of 90.05%, while gB2 had an efficiency of 96.75%. All BALF samples were negative when tested with gB1, and all were positive with gB2 primers (Figure 1). Analysis of gel electrophoresis and melt curves suggests that gB2 is more sensitive without compromising specificity. Lung parenchyma from these mice demonstrated even lower amounts of CMV DNA detectable only with gB2 primers.

Conclusions: Our results suggest that CMV DNA is either continuously shed in the BAL fluid or selectively concentrated in CMV containing cells within the BAL fluid during latency in immune competent hosts. Therefore DNA in BAL samples may not be indicative of CMV activity. Results are dependent in part on assay sensitivity, highlighting the need for standardized assays. Quantification of DNA, changes in cellular composition of BAL, or BAL-RNA may provide additional information.
Role of E2f8 in Tumor Progression, Initiation, and Ploidy in Hepatocellular Carcinoma Using a Tamoxifen-inducible Sa-Cre Murine Model.

Justin T. Huntington, MD

Introduction: The E2F transcription factors are a large family of proteins that regulate cell cycle control that may play a role in carcinogenesis including hepatocellular carcinoma (HCC) and liver disease. E2f8 is an atypical repressor of the cell cycle and plays a role in cell ploidy through regulation of an interesting phenomenon called endocycling in which chromosomes replicate without intervening mitosis or cytokinesis resulting in polyplloid cells. The atypical E2Fs are the least well studied but are known to be important in polyplloidy (E2f8 is necessary for polyplloidy). It remains unclear as to the actual role of E2Fs and polyplpoidy in hepatocarcinogenesis, especially in humans. In the current research, the role of E2f8 in polyplpoidy and tumor progression are evaluated using a murine model.

Methods: A murine model using mixed background male mice with a Tamoxifen-inducible Sa-Cre system was used to downregulate E2f8 activity in a temporal manner. Controls were Cre negative (Cre-) littermates. All mice were given intraperitoneal injections of the hepatocarcinogen diethylnitrosamine (DEN) in a dose of 0.02 mL/g body mass at 20 days of age. Mice were fed Tamoxifen chow at pre-weaning (7 days), early post-weaning (28 days), or late post-weaning (42 days) for 7 days total to evaluate the effects of E2f8 and ploidy in hepatocarcinogenesis. Mice were tailed for genotyping prior to weaning and weaned at 21 days of age. Mice were sacrificed at 9 months of age. Analyses included hepatic mass calculations, flow cytometry to evaluate ploidy, real-time PCR to quantify gene deletion, and histology (H&E).

Results: Deletion of E2f8 was confirmed using real-time PCR of liver samples from Cre+ mice compared to Cre- matches. In the 7-day Tamoxifen group, 87.5% (7 of 8) of Cre+ mice had greater than 10 macroscopic tumors while only 16.7% of the Cre- mice had greater than 10 macroscopic tumors, 66.7% had 1-10 tumors, and 16.7% had no visible tumors at all (n=6). The hepatic mass (liver weight/total body weight) for the Cre+ mice was nearly triple the weight of the Cre- mice (13.34 % versus 5.72%, p=0.01). Despite differences in hepatic mass and tumorigenesis, there were no statistical differences in ploidy as measured by flow cytometry. Tumorigenesis was less marked in the 28-day group with hepatic mass being nearly the same (4.97% for Cre- controls versus 5% for Cre+). However, macroscopic tumors were only visualized in 29.4% of Cre-livers (5 of 17) versus 83.3% of Cre+ samples (10 of 12) and higher tumor numbers were seen in the Cre+ mice. Again, no statistical differences were seen in ploidy levels for the two groups. The 42-day group again demonstrated increased tumorigenesis with only 41.7% of Cre- mice having tumors (5 of 12) while 93.8% of Cre+ mice having tumors (15 of 16). Hepatic mass was slightly increased for Cre+ mice at 6.2% versus 5% for Cre- mice. Again, no statistical difference was seen in terms of ploidy between the two groups.

Conclusions: This current model demonstrates that loss of E2f8 function is important in tumor progression as evidence of increased tumorigenesis regardless of the timing of deletion as compared to appropriate controls. It does appear that early loss of E2f8 is more detrimental in terms of tumorigenesis and loss near the time of DEN injection is less detrimental. Despite other evidence that polyplpoidization may be protective in hepatocarcinogenesis, this study did not reach the same conclusion and raises the possibility that E2f8 may function as a tumor suppressor through a mechanism other than cell ploidy. Illucidating the exact tumor suppressor mechanism of E2f8 is ongoing and needs to be better understood so that possible therapeutics targeting this important transcription factor may be discovered. Results also must be validated in humans. Currently we have IRB approval (OSU-13209) for obtaining human HCC samples with Drs. Schmidt and Bloomston to quantify E2F activity in human HCC. Early results do show significant E2F dysregulation in the human samples.
Engulfment of Apoptotic Cells by Macrophages: A Role of MicroRNA-21 in the Resolution of Wound Inflammation

Amitava Das, M.Pharm; Kasturi Ganesh Barki, MD; Ryan Dickerson; Savita Khanna, PhD; Chandan K. Sen, PhD; Sashwati Roy, PhD

Introduction: At an injury site, efficient clearance of apoptotic cells by wound macrophages or efferocytosis is a prerequisite for the timely resolution of inflammation. Emerging evidence indicates that microRNA-21 (miR-21) may regulate the inflammatory response. In this work, we sought to elucidate the significance of miR-21 in the regulation of efferocytosis-mediated suppression of innate immune response, a key process implicated in resolving inflammation following injury.

Methods: Peripheral Blood Monocytes (PBMCs) were isolated from buffy coats (source leukocytes) obtained from the American Red Cross using Ficoll-Hypaque. The cells were further purified using magnetic cell isolation system and CD14 microbeads (Miltenyi Biotec, Auburn, CA). After isolation, monocytes were seeded in 6-well plates and cultured in RPMI 1640 supplemented with 10% FBS, 1% PSA (penicillin G sodium, streptomycin sulfate, and amphotericin B), 10 μg/ml of polymyxin B, and 20 ng/ml of M-CSF for 5 days in 37°C with 5% CO2. For the Efferocytosis assay, apoptotic Jurkat cells were added to culture plates containing human macrophages at a 1:5 macrophage to Jurkat cell ratio. Prior to co-culture with macrophage, the Jurkat cells were labeled with a red fluorescence cell-tracker reagent. Efferocytosis assay was performed for 1 h at 37°C. Macrophages were washed to remove non-efferocytosed cells.

Results: An increased expression of inducible miR-21 was noted in postefferocytotic peripheral blood monocyte-derived macrophages. Such induction of miR-21 was associated with silencing of its target genes PTEN and PDCD4. Successful efferocytosis of apoptotic cells by monocyte-derived macrophages resulted in the suppression of LPS-induced NF-κB activation and TNF-α expression. Interestingly, bolstering of miR-21 levels alone, using miR mimic, resulted in significant suppression of LPS-induced TNF-α expression and NF-κB activation. We report that efferocytosis-induced miR-21, by silencing PTEN and GSK3β, tempers the LPS-induced inflammatory response. Macrophage efferocytosis is known to trigger the release of anti-inflammatory cytokine IL-10. This study demonstrates that following successful efferocytosis, miR-21 induction in macrophages silences PDCD4, favoring c-Jun–AP-1 activity, which in turn results in elevated production of anti-inflammatory IL-10.

Conclusions: In summary, this work provides direct evidence implicating miRNA in the process of turning on an anti-inflammatory phenotype in the postefferocytotic macrophage. Elevated macrophage miR-21 promotes efferocytosis and silences target genes PTEN and PDCD4, which in turn accounts for a net anti-inflammatory phenotype. Findings of this study highlight the significance of miRs in the resolution of wound inflammation.
P21-Activated Kinase as a Therapeutic Target in Papillary Thyroid Cancer

Kara Keplinger, MD; Chaojie Wang; Sam McCarty, PhD; Samuel Kulp, PhD; Ching-Shih Chen, PhD; Motoyasu Saji, MD, PhD; Matthew Ringel, MD

Introduction: The incidence of thyroid cancer is rising at a faster rate than any solid tumor in the United States, and the number individuals who succumb to thyroid cancer rising annually. The most common mutation in papillary thyroid cancer (PTC) is the BRAF V600E activating mutation, which is associated with aggressive tumor behavior and poor outcome. Treatment options for metastatic PTC remain sparse. Vemurafenib, a direct inhibitor of BRAF V600E has limited efficacy for the treatment of metastatic thyroid cancer, and resistance develops quickly. Interestingly, BRAF activity is required for the activation of p21-activated kinase 1 (PAK1), a kinase involved in metabolism and cell division. PAK1 is upregulated at the invasive fronts of PTC and has a defined role in the epithelial-to-mesenchymal transition. Recent observations from our lab suggest that PAK1 may be involved in the activity or resistance of vemurafenib in thyroid cancer. We have also shown that inhibition of PAK1 with three novel compounds, OSU-03012, YM4, and YM15 results in inhibition of cell migration and anti-proliferative effects in vitro. We hypothesize that PAK1 inhibition is an effective chemotherapeutic strategy in vivo, and may be synergistic in combination with vemurafenib.

Methods: Four human thyroid cancer cell lines, TPC1, C643, BCPAP, and SW1736, have been engineered to stably express luciferase. These cell lines will be used to establish xenografts and to determine therapeutic response of thyroid cancer to PAK1 inhibition with YM4 and YM15 in vivo. To verify that these luciferase-expressing lines behave as their parent lines, cell viability assays were performed with YM4 and YM15. Western blots from time course experiments were also performed to determine the effect of PAK1 inhibition on the expression of important cell signaling regulators, ERK and AKT, as well as vimentin and S6, both downstream targets of PAK1.

Results: Cell viability assays demonstrate reliable cell death between 2.5 and 5 μM of YM4 and YM15. There was no difference between the luciferase-expressing cell lines and the parental lines in the cell viability assays. Minor variations in the sensitivity between cell lines were observed, attributable to differing genetic mutations driving carcinogenesis. Western blot analysis is ongoing.

Conclusions: PAK1 inhibition is active against thyroid cancer in vitro. Future directions include cell viability assays to examine synergy with vemurafenib and murine xenograft modeling to evaluate therapeutic response to PAK1 inhibition.
Knockout of microRNA-21 Leads to Increased Inflammatory Response During Wound Healing

Mithun Sinha, PhD; Subhadip Ghatak, PhD; Amitava Das, M.Pharm; Savita Khanna, PhD; Sashwati Roy, PhD; Chandan K. Sen, PhD

**Introduction:** Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. The wound healing process can be divided into 3 distinct phases. The three phases include the inflammatory phase, the proliferative phase, and the remodelling phase. In the inflammatory phase, neutrophils followed by macrophages infiltrate to the wound area to clear the dead tissues and bacterial debris. Many factors influencing the wound healing process are secreted by macrophages. These include transforming growth factors (TGFs), cytokines and interleukin (IL)–1, tumor necrosis factor (TNF), and platelet derived growth factor (PDGF). MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. miRs have been reported to be involved in various stages of wound healing. They play important role in wound angiogenesis, epithelial-mesenchymal transition among other processes.

**Methods:** We have used a K14 Cre-miR-21fl/fl mice model (resulting in targeted knock out of miR-21 is epithelium of skin). Immunohistochemistry was performed to detect neutrophils, macrophages and fibroblasts. Cytokine array was performed with wound fluids. Western blot was performed using Simon platform.

**Results:** Here using the model mentioned above, we showed that resolution of inflammatory phase gets delayed in wounds of K14 Cre-miR-21fl/fl mice. Immuno-histochemical staining revealed abundance of macrophages at d10 post wounding. Cytokine analysis from the wound fluid of these transgenic mice revealed presence of inflammatory cytokines which are predicted to be regulated by miR-21. The transgenic mice showed compromised quality of healed wounds. Transdifferentiation of one cell type to the other is a very vital process in tissue remodelling. We observed transdifferentiation of macrophages to fibroblast during wound healing and found that this transdifferentiation was hampered in K14 Cre-miR-21fl/fl mice.

**Conclusions:** We hypothesize that miR-21 plays an important role by governing the quality of healing during the process of wound healing. Further studies are being carried out to elucidate the biological pathways for miR-21 mediated wound healing.