17th Annual Surgery Research Conference

Richard M. Ross Heart Hospital Auditorium
Friday, May 25, 2012
DEPARTMENT OF SURGERY
THE OHIO STATE UNIVERSITY

17TH ANNUAL SURGERY RESEARCH CONFERENCE

RICHARD M. ROSS HEART HOSPITAL AUDITORIUM
FRIDAY, MAY 25, 2012
Welcome

Welcome to the 17th 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 residents, graduate students and postdoctoral research trainees to develop their scientific communication skills. Each year Dr. Christopher Ellison, Chair of the Department of Surgery, invites a leader in Surgery to visit 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. Stanley Ashley, Chief Medical Officer and Senior Vice President for Medical Affairs at Brigham and Women’s Hospital and the Frank Sawyer Professor of Surgery at Harvard Medical School, as our guest. In addition to the visiting professor, we also ask a prominent research leader at Ohio State University to participate in our conference by serving on a panel of faculty judges. Dr. Charles Lockwood, Dean of The Ohio State University College of Medicine has generously agreed to participate.

Over the years the format for the conference has developed into 2 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. This year for the first time we invited 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 Masters 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 Bumgardner, MD, PhD
Agenda
Friday, May 25, 2012

Welcome and Introduction of Visiting Professor
Welcome and introduction by E. Christopher Ellison, MD, the Robert M. Zollinger Professor and Chairman of Surgery, CEO of the Faculty Group Practice, Associate Vice President for Health Sciences, Vice Dean of Clinical Affairs, The Ohio State University College of Medicine

Introduction to the Conference
Conference purpose and format by Ginny L. Bumgardner, MD, PhD, Professor of Surgery, Division of Transplantation, Associate Dean for Research Education, Director, Master’s of Medical Science Program, The Ohio State University College of Medicine

Judges
Stanley W. Ashley, MD, E. Christopher Ellison, MD, and Charles J. Lockwood, MD

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

Session 1: Oral Presentations, 8:15 to 9:45 a.m.
Influence of obesity on groin surgical site infections after vascular surgery. Laura A. Peterson, MD • Faculty Advisor: Jean Starr, MD • Discussant: Ahmet Kilic, MD .................................................................11

MicroRNA-1 induction in ischemic wounds attenuates keratinocyte migration via an aquaporin-3 mediated pathway. Jaideep Banerjee, MS • Faculty Advisor: Chandan K. Sen, PhD • Discussant: John Phay, MD .........................12

Engulfment of apoptotic cells induces miR-21 expression and switches human macrophages from a pro-inflammatory to anti-inflammatory phenotype. Amitava Das, PharmD • Faculty Advisor: Sashwati Roy, PhD • Discussant: Charles Cook, MD .................................................................13

Targeting Aperrent Epigenetic Modification by Circulating Microvesicles During Sepsis. Jon Wisler, MD • Faculty Advisor: Clay B. Marsh, PhD • Discussant: Steven Steinberg, MD .................................................................14

A new paradigm for the regulation of antibody production in a transplant model. Jason Zimmerer, PhD • Faculty Advisor: Ginny L. Bumgardner, MD, PhD • Discussant: William Carson, MD .................................................................15

HB-EGF Protects the Intestines from Radiation Therapy-Induced Intestinal Injury. Mika Matthews, MD • Faculty Advisor: Gail Besner, MD, MBA • Discussant: Sashwati Roy, PhD ..............................................................................16
Break and Poster Presentations, 9:45 to 10:30 a.m.
First evidence of biofilm in deep sternal wound infection. Haytham Elgharably, MD • Faculty Advisor: Chandan K. Sen, PhD.................................17

It Takes More Than One miRNA to Treat HCC. Jon Henry, MD • Faculty Advisor: Thomas D. Schmittgen, PhD..................................................18

A Differential MicroRNA Profile Distinguishes Cholangiocarcinoma from Pancreatic Adenocarcinoma. Amy Collins, MD, MS • Faculty Advisor: Mark Bloomston, MD.................................................................19

Novel Large-Animal Model to Study Acute Myocardial Infarction Using Platelet-Induced Thrombus. Tyler Spata, MD • Faculty Advisor: Chittoor B. Sai-Sudhakar, MD ..................................................20

Immunologically Modified FETs for Protein Detection in Physiologic Buffers. Andrew Theiss, BS • Faculty Advisor: Gregg A. Hadley, PhD .........................21

Symptomatic and Radiographic Evaluation of Hiatal Hernia Recurrence Following Laparoscopic Paraesophageal Hernia Repair with Polyester Composite Mesh Reinforcement. Mark Wendling, MD • Faculty Advisor: Kyle Perry, MD..............................................22

Session 2: Oral Presentations, 10:30 to 12 noon
MicroRNA Profiling of Problematic Melanocytic Lesions. Sara Martin del Campo, MD • Faculty Advisor: William E. Carson, III, MD • Discussant: Mark Bloomston, MD.................................................................23

A Scoring System for Prognosis and Treatment of Malignant Bowel Obstruction. Jon Henry, MD • Faculty Advisor: Mark Bloomston, MD • Discussant: Kyle Perry, MD..........................24

Concomitant dysregulation of miR-151-3p and miR-126 correlates with improved survival in resected cholangiocarcinoma. Megan E. McNally, MD • Faculty Advisor: Mark Bloomston, MD • Discussant: Pete Muscarella, MD ................................25

Proteasome Inhibition Induces Apoptosis of Melanoma Cells. Eric A. Luedke, MD • Faculty Advisor: William E. Carson, III., MD • Discussant: Doreen Agnese, MD.................................................................26

Pharmacological Modulation of PTEN Ameliorates the Progression of Pulmonary Hypertension in Heart Failure. Yazhini Ravi, MD • Faculty Advisor: Chittoor B. Sai-Sudhakar, MD • Discussant: Daniel Eiferman, MD ..........27

Porcine wet lab improves surgical skills in third year medical students. Joseph Drosdeck, MD • Faculty Advisor: Peter Muscarella, MD • Discussant: Amer Rajab, MD.................................................................28
Visiting Professor

Stanley W. Ashley, MD

Stanley W. Ashley, MD, is Chief Medical Officer and Senior Vice President for Medical Affairs at Brigham and Women's Hospital as well as the Frank Sawyer Professor of Surgery at Harvard Medical School.

A graduate of Oberlin College and Cornell University Medical College, he completed a residency in General Surgery and joined the faculty at Washington University in St. Louis. He subsequently spent 7 years at the University of California at Los Angeles until 1997 when he assumed the position of Vice Chairman of the Department of Surgery and Program Director of the General Surgery Residency at Brigham and Women's Hospital as well as his current position at Harvard Medical School.

Dr. Ashley is a gastrointestinal surgeon whose primary interests are diseases of the pancreas and inflammatory bowel disease. His focus is on practical aspects of measurement of surgical quality and how these can be applied to improve outcomes, particularly for the individual surgeon. Closely related to this, he has an interest in surgical education, both at the graduate and postgraduate (MOC) levels, and its integration into a certification/recertification process that ensures quality of care.

His research, which has been funded by both the Department of Veterans Affairs and the National Institute of Health, has examined the pathophysiology of the small bowel and pancreas. He is the author of more than 300 publications. He serves on numerous editorial boards, including the Journal of Gastrointestinal Surgery, the Journal of the American College of Surgeons, Current Problems in Surgery, and ACS Surgery. He is currently the President of the American Board of Surgery and Secretary-Elect of the Society for Surgery of the Alimentary Tract.
Invited OSU College of Medicine Faculty Judge

Charles J. Lockwood, MD, MHCM

Charles J. Lockwood, MD, MHCM, is Vice President for Health Sciences at The Ohio State University and Dean of the College of Medicine. Prior to this position he was the Anita O’Keeffe Young Professor and chair of Obstetrics, Gynecology and Reproductive Sciences at the Yale School of Medicine, chief of Ob/Gyn at Yale-New Haven Hospital, and chair of the Yale Medical Group Board of Governors. He also previously served as chair of Ob/Gyn at the New York University School of Medicine.

Lockwood graduated magna cum laude from Brown University, earned his medical degree at the University of Pennsylvania, and received a master’s in healthcare management from the Harvard School of Public Health. He completed a residency in Ob/Gyn at Pennsylvania Hospital and fellowships in maternal-fetal medicine and coagulation at Yale-New Haven Hospital and Mount Sinai School of Medicine, respectively.

With continuous NIH funding for more than 20 years, Lockwood has 245 peer review publications. He has authored or co-authored three monographs/books, co-edited seven obstetrical textbooks, and is section editor for obstetrics for UpToDate and editor-in-chief of Contemporary OB/GYN, having won four national publishing awards for his editorials. Former treasurer and president of the Society for Gynecological Investigation, Lockwood has chaired various American College of Obstetricians and Gynecologists committees, served on multiple NIH Study Sections and FDA committees. Routinely named a top doctor by New York Magazine and Castle Connolly, he is a member of the Alpha Omega Alpha honor society and was inducted into the Institute of Medicine in 2010.
Presenters

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

Amy Collins, MD, MS
General Surgery Resident
Hometown: West Palm Beach, FL
BS: Psychobiology, Florida Atlantic University, Boca Raton, FL
MD: The University of South Florida College of Medicine, Tampa, FL
Additional training: Master of Medical Science, The Ohio State University, Columbus, OH
Research interests: Dysregulation of microRNAs and the role of the tumor microenvironment in gastrointestinal cancers.

Amitava Das, M.Pharm
Graduate Research Associate
Hometown: Kolkata, India
B. Pharmacy: PES College of Pharmacy, Bangalore, India
Additional training: M. Pharmacy, PES College of Pharmacy, Bangalore, India
Research interest: Wound healing, Inflammation, MicroRNA,

Joseph Drosdeck, MD
General Surgery Resident
Hometown: Milwaukee, WI
BA: Psychology, University of Wisconsin - Milwaukee, WI
MD: University of Wisconsin, Madison, WI
Research Interests: Minimally invasive surgery, surgical education

Haytham Elgharably, MD
Post Doctoral Researcher
Hometown: Gharbeya, Egypt
MD: Zagazig University, Egypt
Additional training: Cardiothoracic surgery residency, National Heart Institute, Cairo, Egypt
Research interest: Post-sternotomy pain, sternotomy wound infection, spinal cord ischemia with aortic surgery.
Presenters

Jon Henry, MD
General Surgery Resident, Master of Medical Science Program Candidate
**Hometown:** Geneva, Ohio
**BS:** Biology, Ohio State University, Columbus, OH
**MD:** Ohio State University, Columbus, OH

Eric Luedke, MD
General Surgery Resident, Master of Medical Science Program Candidate
**Hometown:** Milwaukee, WI
**BS:** Marquette University, Milwaukee WI
**MD:** Medical College of Wisconsin, Milwaukee, WI
**Research interests:** Colorectal/MIS, cancer immunology

Sara Martin del Campo, MD
General Surgery Resident, Master of Medical Science Program Candidate
**Hometown:** Peoria, IL
**BS:** Biology, Indiana University, Bloomington, IN
**MD:** The University of Iowa Carver College of Medicine, Iowa City, IA
**Research interest:** MicroRNA expression in melanoma.

Mika Matthews, MD
General Surgery Resident
**BS:** University of Michigan, Ann Arbor, MI
**MD:** Morehouse School of Medicine, Atlanta GA
**Research Interest:** Intestinal inflammation and repair

Megan McNally, MD
Surgical Oncology Fellow, Master of Medical Science Program Candidate
**Hometown:** Joplin, MO
**BLA:** University of Missouri-Kansas City, Kansas City, MO
**MD:** University of Missouri-Kansas City, Kansas City, MO
**Additional training:** General Surgery Residency, University of Missouri-Kansas City, Kansas City, Missouri, July 2005-June 2010
**Research interests:** Gastrointestinal Surgical Oncology, outcomes research in gastrointestinal surgical oncology
Presenters

Laura Peterson, MD
General Surgery Resident, Master of Medical Science Program Candidate
BS: Zoology, University of Wisconsin, Madison, WI
MD: University of Wisconsin School of Medicine and Public Health
Research Interests: Alpha tocotrienol and acute ischemic stroke, cerebral ischemia, venous thrombosis

Yazhini Ravi, MD
Post Graduate Researcher
Hometown: Chennai, India
MD: Sri Ramachandra University, India
Research interest: Pulmonary hypertension and heart failure

Tyler Spata, MD
General Surgery Resident, Master of Medical Science Program Candidate
BBA: Business Administration, The University of Texas, Austin, TX
MD: The University of Texas Medical Branch, Galveston, TX
Additional training: Cardiovascular Research, The University of Texas Health Science Center, Houston, TX; Cardiovascular Surgery/Anesthesia Research, Texas Heart Institute, Houston, TX
Research interest: Cardiac ischemia, cardiac transplantation, mechanical cardiac-assist devices, and revascularization techniques.

Andrew Theiss, BS
Research Associate
Hometown: Westerville, Ohio
BS: Electrical Engineering, The Ohio State University, Columbus, OH
Research interest: Biosensors, FET-based sensors, nanotechnology, medical devices

Mark Wendling, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Mayville, WI
BA: Biology, Lawrence University, Appleton, WI
MD: Medical College of Wisconsin, Milwaukee, WI
Research interest: Minimally invasive surgery
Presenters

Jon Wisler, MD
General Surgery Resident, Masters of Medical Science Program Candidate
Hometown: Westerville, OH
BA: Microbiology, Miami University, Oxford, OH
MD: University of Cincinnati, Cincinnati, OH
Research Interest: Epigenetic regulation in sepsis and inflammation

Jason Zimmerer, PhD
Postdoctoral Fellow
Hometown or birthplace: Zanesville, OH
BS: Biochemistry, Ohio Northern University, Ada, OH
Graduate Program: Integrated Biomedical Science Graduate Program, The Ohio State University, Columbus, OH
Additional training: Postdoctoral fellowship, The Ohio State University, Columbus, OH
Research interest: Transplant immunology, cancer immunology, molecular genetics
Influence of Obesity on Groin Surgical Site Infections After Vascular Surgery

Laura A Peterson MD and Jean Starr MD

Objective: Surgical site infections are a significant risk after vascular surgical procedures and can be due to a multitude of contributing factors. The purpose of this study was to examine the effect of obesity on the infection rates of vascular surgery patients undergoing groin incisions and evaluate other confounding factors.

Methods: A single center retrospective cohort study was undertaken to examine all vascular surgery patients undergoing groin incisions for femoral artery vascular procedures from February 2007 through August 2008. Patient demographics, characteristics, including BMI, and potential confounding variables of diabetes, hypertension, smoking, renal insufficiency, coronary artery disease, cerebrovascular disease, pulmonary disease, and hyperlipidemia were recorded. All patients were monitored for infection for 30 days and those having a prosthetic implant were monitored for one year.

Results: A total of 451 patients were included in this study. 6% (n=29) of the patients developed groin wound infections diagnosed by clinical (NSQIP criteria) or microbiologic wound culture criteria. The most common causative organism was MRSA (n=9). Patients with surgical site infections had statistically higher BMIs (30 vs. 27 p=0.016). Further analysis demonstrated that the surgical site infection group had a higher percentage of obese individuals than did the infection-free group (45% vs 26%, p=0.009). Of the other variables examined, only diabetes was associated with increased surgical site infections (p=0.007).

Conclusions: Obesity, as defined by a BMI greater than 30, and the presence of diabetes independently increase the chance of post operative groin wound infections in the vascular surgery patient population. These patients may warrant better pre-op preparation, closer post-operative care, and modification of surgical technique in order to avoid an infectious complication.
MicroRNA-1 Induction in Ischemic Wounds Attenuates Keratinocyte Migration via an Aquaporin-3 Mediated Pathway.

J. Banerjee¹, Hussain S-R¹, S. Biswas¹, T. Schmittgen², S. Khanna¹, S. Roy¹, C.K Sen¹

¹Comprehensive Wound Center, Department of Surgery, ²College of Pharmacy, The Ohio State University, Columbus, OH 43210

Introduction: Ischemia is a key factor that limits dermal wound healing. To understand and characterize the biology of ischemic wounds, we have developed a murine model of ischemic wound healing. In this study we have used this model to identify the molecular mechanisms of gene regulation that result in impaired healing in ischemic wounds. MicroRNAs have been reported to play an important role in regulating gene expression in ischemic wounds. Recent studies show that aquaporin 3 (AQP3) serves as facilitator of keratinocyte migration.

Methods & Results: AQP3 knockdown by siRNA in HacaT cells resulted in slower cell migration as assayed by a scratch assay (n=4, p<0.05%). We observed that AQP3 mRNA and protein expression is induced in non-ischemic wounds as compared to unwounded skin while this induction is not observed in ischemic wounds (n=4, p<0.05%). To identify AQP3 gene regulation by microRNAs, a microRNA profiling was done between skin, non-ischemic and ischemic wound samples. Only 19 microRNAs were found to be down regulated and 6 microRNAs were found to be up regulated in ischemic wounds as compared to non-ischemic wounds (n=3, p<0.05%). Among these microRNAs, miR-1 was found to be elevated and we found that miR-1 induction resulted in down regulation of AQP3 mRNA and protein expression (n=3, p<0.05%). miR-1 induction using miR-1 mimic in HacaT cells was also found to result in slower cell migration as assayed by a scratch assay (n=4, p<0.05%).

Conclusions: Studies are currently in progress to determine mechanisms of AQP3 down regulation by miR-1 induction, resulting in impaired cell migration in ischemic wounds.
Engulfment of Apoptotic Cells Induces miR-21 Expression and Switches Human Macrophages from a Pro-Inflammatory to Anti-Inflammatory Phenotype

Amitava Das, Kasturi Ganesh, Ryan Dickerson, Savita Khanna, Chandan K. Sen and Sashwati Roy

Introduction: Efferocytosis (engulfment of apoptotic cells) by macrophages is the final step in the cell-death program and drives macrophages to an anti-inflammatory state by inducing production of the anti-inflammatory cytokines and suppressing the pro-inflammatory counterparts. Emerging studies indicate that miR-21 plays a key role in regulating proteins that orchestrate the inflammatory process. The objective of the current study was to investigate whether miR-21 plays a role in the efferocytosis-induced switching of macrophages to anti-inflammatory state.

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 ìg/ml of polymyxin B, and 20 ng/ml of M-CSF for 5 days in 37°C with 5% CO₂. For the Phagocytosis 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. Phagocytosis assay was performed for 1 h at 37°C. Macrophages were washed to remove non-phagocytosed cells.

Results: LPS treatment of human macrophages resulted in a time dependent increase in miR-21 expression. Such increase was further potentiated (~1.5 fold, 6 hours and ~2 folds, 24 hours post LPS stimulation; p<0.05; n=4) by efferocytosis. The tumor suppressor Phosphatase and tensin homolog (PTEN), a direct target of miR-21, was significantly downregulated (p<0.05; n=3) following engulfment of apoptotic cells compared to controls (macrophages co-cultured with viable cells) demonstrating that the silencing of this proteins is due to increased miR-21 expression. Efferocytosis suppressed the secretion of LPS-induced TNF-α, a pro-inflammatory cytokine by macrophages. Delivery of miR-21 (via miR-21 mimic) to human primary macrophages downregulated PTEN. We further show that changing PTEN levels in cells directly affect NF-κB activation. NF-κB is known to regulate TNF-α gene transcription.

Conclusion: Taken together these data demonstrate a novel miR-21-PTEN dependent mechanism of suppression of NF-κB activation and subsequent TNF-α production. Such inhibition of pro-inflammatory TNF-α may be involved in efferocytosis mediated resolution of inflammation.
Targeting Aberrent Epigenetic Modification by Circulating Microvesicles During Sepsis

Jon R Wisler, Duaa Dakhallah, Melissa G. Piper, Clay B. Marsh

**Introduction:** Sepsis is a severe disease with high mortality and significant morbidity. Following sepsis, the compensatory anti-inflammatory response (CARS) causes global immunosuppression that predisposes survivors to increased risks of infections from opportunistic infections. Studies suggest that epigenetic regulation of key inflammatory mediators including TNF-α and IL-1α may be responsible for the immunosuppressed phenotype observed. *In vitro* evidence suggests promoter methylation changes of TNF-α and IL-1β, mediated through DNA methyltransferases (DNMTs), may be responsible for this immunosuppression; however, no systemic mechanism has been identified. Microvesicles (MV) are small membrane-bound structures capable of transmitting genomic and protein information between distant cells. We have observed that circulating microvesicle (MV) production and function is altered in sepsis. Our central hypothesis is DNMT mRNA expression increases in the plasma MVs of patients with sepsis and that these mRNA transcripts are transferred to naïve monocytes and elicit an immunosuppressed phenotype due to DNA methylation. The overall objective was to confirm DNMT mRNA expression in circulating MVs and characterize the effects of mRNA transfer on naïve recipient cells.

**Methods:** Circulating MVs were isolated from plasma of patients with sepsis on days 1, 3 and 5 after diagnosis. mRNA expression was characterized by RT-PCR for DNMT-1, 3a, and 3b. Naïve human monocytes were treated with MVs isolated from patients with sepsis and critically ill, non-septic controls. Gene expression and TNF-α promoter methylation were assessed using PCR. Immunosuppression was tested by subsequent stimulation with endotoxin. Pro- and anti-inflammatory cytokines data were analyzed with Bio-Rad bioplex assays.

**Results:** Sepsis derived MVs contained increases in DNMT 1, 3a, and 3b mRNA. Monocytes treated with sepsis derived MVs increased expression of all DNMTs. In addition, TNF-α promoter methylation increased after treatment. When stimulated with endotoxin, monocytes pretreated with sepsis derived MVs demonstrated a reduction in TNF-α generation and immunosuppressed phenotype. The methylation pattern and TNF-α suppression was dose dependent and could be alleviated by pharmacologic methylation inhibitors or titration with MVs derived from healthy individuals.

**Conclusions:** These data demonstrate that circulating MVs are an important mediator of epigenetic modification and immunosuppression during sepsis. These regulators are able to be transferred from MVs to naïve cells suggesting a role in systemic communication and disease progression. The ability of MVs derived from healthy individuals and methylation inhibitors to alleviate methylation at TNF-α and maintain the immunocompetent TNF-α response suggests targeting of circulating MVs may be used as a novel treatment strategy in patients with sepsis to prevent the deleterious effects of the CARS response and post-sepsis immunosuppression.
A New Paradigm for the Regulation of Antibody Production in a Transplant Model

Jason M. Zimmerer, Thomas A. Pham, Virginia M. Sanders, Ginny L. Bumgardner

Introduction: Clinical and experimental data indicate that transplant-specific antibodies (alloantibodies) play a critical role in acute and chronic rejection after transplant. While it is well known that CD4+ T cells and B cells collaborate for antibody production, our group has recently reported that CD8+ T cells downregulate alloantibody responses following transplantation. However, the exact mechanism of CD8-mediated inhibition of alloantibody levels remains unclear. We have also reported that alloantibody production is enhanced in perforin KO and FasL KO transplant recipients. Therefore, we hypothesized that alloprimed CD8+ cytotoxic T cells downregulate antibody production by killing antibody producing B cells.

Methods: We adoptively transferred wild-type, FasL-deficient, or perforin-deficient CD8+ T cells into CD8 KO hepatocyte recipients on the day of transplant. Serum alloantibody levels were measured on day 14 post-transplant. Additionally, we utilized an in vivo and in vitro cytotoxicity assays to investigate whether alloprimed IgG1+ B cells are preferentially cleared by CD8-dependent mechanisms.

Results: CD8+ T cells utilize cytotoxic molecules to inhibit antibody production. We found that adoptive transfer of wild-type (WT) CD8+ T cells into CD8 KO recipients significantly reduced serum alloantibody compared to untreated CD8 KO recipients. However, adoptive transfer of CD8+ T cells which lacked cytotoxic effector molecules, Fas L mutant or perforin KO, were less effective in reducing alloantibody in CD8 KO recipients. CD8+ T cells utilize cytotoxic molecules to eliminate antibody producing B cells. Utilizing an in vivo cytotoxicity assay, we found that alloprimed IgG1+ (antibody producing) B cells are preferentially killed in WT (CD8-sufficient) as compared to CD8 KO (CD8deficient) recipients. CD8 KO recipients reconstituted with FasL KO CD8+ T cells or perforin KO CD8+ T cells had significantly reduced cytotoxicity towards IgG1+ B cells as compared to recipients receiving WT CD8+ T cells. CD8+ T cells directly kill antibody producing B cells. A 4 hour in vitro cytotoxicity assay exhibited that alloprimed IgG1+ B cells underwent apoptosis when co-cultured with bulk alloprimed CD8+ T cells but not when co-culture with naïve CD8+ T cells or bulk third party activated CD8+ T cells.

Conclusion: This data is consistent with the interpretation that alloprimed CD8+ T cells downregulate posttransplant alloantibody production by the use of specific cytotoxic effector molecules for direct elimination of alloprimed IgG1+ B cells.
HB-EGF Protects the Intestines from Radiation Therapy-Induced Intestinal Injury

Mika A.B. Matthews, MD, Daniel Watkins, MD, Amanda Darbyshire, William E. Carson III, MD, Gail E. Besner, MD

Introduction: Every year nearly one million cancer patients are at risk for developing acute and/or chronic radiation enteritis after undergoing radiation therapy (RT). Toxic exposure to radiation inhibits mucosal crypt cell proliferation and causes intestinal epithelial cell (IEC) apoptosis, causing ischemia, ulceration, and necrosis of the intestinal lining. We have previously shown that heparin-binding EGF-like growth factor (HB-EGF) downregulates pro-inflammatory cytokine expression, reduces reactive oxygen species production, and protects the intestines from several different forms of intestinal injury. The objective of our study is to evaluate how HB-EGF affects radiation therapy-induced intestinal injury.

Methods: Adult female BDF₁ mice were given IP injections of HB-EGF (800 µg/kg) or the same volume of phosphate buffered saline (PBS) once daily for 3 days, followed by total body irradiation (TBI) with a dose of 10 Gy. For crypt proliferation studies, animals were injected with BrdU (30 mg/kg IP) 69 hours after radiation-therapy, and sacrificed 72 hours after radiation-therapy. BrdU immunohistochemistry of intestinal tissue sections was used to identify proliferative mucosal crypt cells. Crypts containing 3 or more BrdU positive cells were considered to be proliferative crypts. To determine gut barrier function, intestinal permeability was examined by administering FITC-dextran via gavage 4 days after TBI, followed by serum collection 4 hours later for determination of serum FITC-dextran levels. Segments of the ileum and descending colon/rectum were harvested immediately after serum collection to evaluate histologic injury using H&E staining and a radiation injury grading scale. Two sections per intestinal segment were blindly scored by two independent investigators.

Results: As determined by BrdU immunohistochemistry, we found that exposure to RT significantly decreased the mean percentage of proliferative mucosal crypts in all segments of the small intestine compared with non-irradiated intestine (p<0.05). However, the pre-treatment of irradiated mice with HB-EGF significantly increased the percentage of proliferative crypts compared with non-HB-EGF-treated irradiated mice in the duodenum (79.2% ± 6.8 vs. 55.1% ± 9.3, p<0.001), jejunum (75.7% ± 11.2 vs. 54.1% ± 9.9, p<0.005), and ileum (63% ± 14.1 vs. 42.2% ± 8.4, p<0.05). Exposure to RT led to significant intestinal histologic injury compared with non-irradiated intestine (p<0.005). However, the pre-treatment of irradiated mice with HB-EGF decreased the severity of histologic injury compared with non-HB-EGF-treated irradiated mice in the ileum (2.8 ± 0.8 vs. 3.25 ± 0.7, p<0.005) and descending colon/rectum (1.1 ± 0.3 vs. 1.6 ± 0.7, p<0.005). Using spectrophotofluorometry, plasma FITC dextran levels were significantly higher in mice exposed to RT compared with plasma levels in uninjured mice (0.09 µg/mL ± 0.02 vs. 0.04 µg/mL ± 0.005, p<0.01). However, HB-EGF pre-treatment resulted in a decrease in plasma FITC dextran levels compared with non-HB-EGF-treated irradiated mice (0.07 µg/mL ± 0.02 vs. 0.09 µg/mL ± 0.02, p=0.08).

Conclusions: These results provide evidence that administration of exogenous HB-EGF preserves mucosal crypt cell proliferation, reduces intestinal histologic injury, and maintains gut barrier function after exposure to radiation therapy. Administration of HB-EGF may represent a novel therapy for the prevention of radiation therapy-induced intestinal injury.
First Evidence of Biofilm in Deep Sternal Wound Infection

Haytham Elgharably, MD, Hamdy Awad, MD, Ethan Mann, PhD, Sashwati Roy, PhD, Gayle Gordillo, MD, Daniel Wozniak, PhD, Chittoor B. Sai-Sudhakar MD, Chandan K. Sen, PhD

Introduction: Deep sternal wound infection (DSWI) is a serious complication after cardiac surgery with high morbidity and mortality. Management of DSWI requires invasive procedures such as, sternal debridement and primary closure or sternectomy with myocutaneous flap reconstruction along with use of broad spectrum antibiotics. Failure of standard antimicrobial therapy in controlling DSWI is a common clinical concern. The purpose of this pilot study is to investigate the existence of biofilm in patients with DSWI. A biofilm is complex microbial community in which bacteria attach to a biological (e.g. wound tissues) or non-biological (e.g. stainless steel wires) surface and are embedded in a self-produced extracellular polymeric substance. Biofilm related infections represent a major clinical challenge with resistance to both host immune defenses and standard antimicrobial therapies.

Methods: Candidates for this pilot study were patients scheduled for a debridement procedure of an infected sternal wound after a cardiac surgery. Four patients were recruited in the study. All cases had marked dehiscence of all layers of the wound down to the sternum with no signs of healing after more than 30 days of receiving broad spectrum antibiotics post-surgery. Microbiological analysis of wound discharge revealed *Staphylococcus aureus* colonization of the wounds. After consenting patients, debrided tissue and extracted stainless steel wires were collected during the debridement procedure.

Results: Debrided tissues were examined by gram stain which showed large aggregations of gram positive cocci. Immune-fluorescent staining of the debrided tissues using a specific antibody against *Staphylococcus* demonstrated the presence of thick clumps of *Staphylococcus* colonizing the wound bed. Scanning of tissue samples with scanning electron microscope showed aggregates of cocci attached to the wound surface. More interestingly, scanning of the extracted wires showed attachment of these cocci aggregation to the wire metal surface.

Conclusion: These observations along with the clinical presentation of the patients and failure of antibiotics to control the infection, provides first evidence that supports the presence of biofilm in these cases. Clinical introduction of the biofilm infection concept in DSWI will change the current management strategies from standard antimicrobial therapy to anti-biofilm strategy, which eventually will help to improve the clinical outcomes.
It Takes More Than One miRNA to Treat HCC

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Introduction: The involvement of microRNA (miRNA) in solid malignancies has been well detailed over the past decade. One focus of miRNA research is their application for the care of oncology patients. In Hepatocellular Carcinoma (HCC), a unique miRNA signature has been described by us and others in which miRNAs Mir-199a-3p and Mir-221 are commonly disregulated. We have demonstrated that targeting the cell surface protein, CD44, miR-199a-3p decreases the proliferation and invasion of HCC in CD44-prominent cell lines. Our lab has also demonstrated that antisense inhibition of miR-221 in vivo prolongs the survival of mice xenografted with HCCs. It is assumed that single miRNA replacement or inhibitory therapy for a cancer will not be effective on every patient. Conceivably, a combination approach would have a broader spectrum of susceptible tumors and improved efficacy in patients. Therefore we hypothesized that combination therapy of miR-199a-3p oligo mimic, anti-miR-221 oligo, and sorafenib would have an increased spectrum of susceptibility in cell lines and potentially have increased efficacy.

Methods: Despite the demonstration of anti-miR-221’s effectiveness in vivo, it’s effect on multiple HCC cell lines has not been studied in vitro. Therefore, we first conducted anti-proliferation analysis of anti-miR-221 on 6 HCC cell lines. IC50s were calculated for all cell lines that were susceptible to each compound. Proliferation was then analyzed with regards to combination therapy of anti-miR-221 and sorafenib, miR-199a-3p and sorafenib, and anti-miR-221. The effects were then analyzed for synergy.

Results: Anti-miR221 treatment (100 nM) on six HCC cell lines reduced proliferation by 20% in only two of the cell lines: Hep 3B (21% reduction), and Hep G2 (25% reduction). All 6 cell lines were susceptible to sorafenib therapy with IC50s as follows: HepG2 11.3 uM, Hep3B 15.3uM, PLC/PRF5 11.3 uM, SNU182 15.3 uM, SNU423 19.4 uM, and SNU 449 uM. Combination therapy with miR-199a-3p (50nM) and sorafenib (20 uM) was conducted in SNU423 and Snu449 cells. Sorafenib and miR-199a-3p mimic reduced proliferation by 22% and 97% in the SNU423 and SNU449 cells, respectively, compared to control oligo. The combination of anti-miR-221 and sorafenib in HepG2 and SNU423 cells reduced proliferation by 40% and 20%, respectively, compared to the control oligo while the SNU449 treated cells were unchanged by sorafenib and anti-miR-221.

Conclusions: In HCC, the regulation of miR-199a-3p or miR-221 with oligo drugs alone is unlikely to be a benefit for all patients. Sorafenib increases the anti-proliferative activity of miR-199a-3p mimic, however, this occurs only in cells that are susceptible to the mimic alone. Further studies pinpointing the activity of miRNA therapy with conventional anti-cancer agents such as sorafenib will allow us to determine if the combination is additive or synergistic. These preliminary results warrant further investigation of combining miRNA therapeutics and traditional anticancer agents for selective, personalized medicine.
A Differential MicroRNA Profile Distinguishes Cholangiocarcinoma from Pancreatic Adenocarcinoma

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Introduction: Cancers of the bile duct and the pancreas are virtually indistinguishable using conventional histopathological and clinical characteristics. We sought to utilize microRNA (miR) profiling to differentiate these two cancers.

Methods: RNA was harvested from the tumors of patients undergoing curative resection for cholangiocarcinoma (n=75) or pancreatic adenocarcinoma (n=20) and compared with adjacent normal bile duct or pancreas, respectively. Differential microRNA expression profiles were determined using NanoString technology.

Results: 41 differentially expressed miRs were identified in cholangiocarcinoma (25 overexpressed, 16 underexpressed) and 52 miRs were found in pancreatic adenocarcinoma (30 overexpressed, 22 underexpressed) relative to adjacent normal tissue. Of these two profiles, 15 miRs were commonly dysregulated between tumor types. Eight miRs were similarly over- or underexpressed in cholangiocarcinoma and pancreatic adenocarcinoma whereas the other seven miRs had inverse expression levels.

Conclusions: Cholangiocarcinoma has a distinct miR profile from pancreatic adenocarcinoma. Discrimination between these two tumor types may be possible with as few as seven miRs.
Novel Large-Animal Model to Study Acute Myocardial Infarction Using Platelet-Induced Thrombus

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Introduction: Myocardial infarction is the leading cause of mortality worldwide. As the proportion of elderly in the world increases, a better understanding of myocardial infarction and the resultant propagation of the cardiac inflammatory cascade is pivotal. In humans, acute myocardial infarction is a result of thromboembolic events. Various animal models have been created in order to better study acute myocardial injury; however, these animal models do not mimic the thromboembolic events which occur in humans. Platelets have an integral role in triggering the initial inflammatory reaction in vascular injury. Through injection of an autologous platelet-induced thrombus into a specific area of the heart through heart catheterization, one can create a better animal model to study the acute myocardial inflammatory events after ischemic cardiac injury.

Methods: Autologous platelets from sheep (n=8) were prepared to create a thrombus. Under fluoroscopy and continuous cardiac telemetry, the thrombus was injected into the left circumflex artery to create an acute myocardial infarction. Blood was collected prior to embolization as well as post-procedure day # 1, 2 and 3. The sheep myocardium was harvested on post-procedure day #3 for further investigation.

Results: Myocardial infarction via ST elevations and prolonged QRS were noted in all of the animals. Completion angiograms of the sheep showed occlusion of the microvasculature compared to pre-embolization fluoroscopy. An immediate rise in troponin levels (>150 ng/mL) were observed over the 3-day course post-procedure. At tissue analysis, fibrosis along the lateral and basal areas of the heart corresponding to the circumflex distribution was noted.

Conclusions: An autologous platelet-induced thrombus model can be used to create an acute myocardial injury is an ovine model. This unique model has the potential to serve as a platform to better characterize the local and global sequelae of the thromboembolic myocardial infarction that occurs in humans.
Immunologically Modified FETs for Protein Detection in Physiologic Buffers

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Introduction: Field effect transistors (FETs) are solid-state electrical devices with semiconductor channels through which charge carriers are able to migrate and generate current. FETs can be modified to allow for protein sensing by deployment of affinity elements (antibodies) as receptors on the semiconducting channel surface to create an immunologically modified FET (immunoFET). Binding of analyte to the receptors causes modulation of FET properties (charge carriers, or current) that are easily detectable, allowing for quantitative detection of unlabeled protein analytes. These FET architectures are economical, scalable, and allow for real-time, point-of-care diagnostic use. We present the detection of multiple inflammatory protein analytes (monokine induced by interferon gamma (MIG, CXCL9), IP-10, RANTES) in physiologic buffers, proving the feasibility of immunoFET operation in physiologic conditions.

Methods: The surfaces of AlGaN/GaN heterojunction FETs were modified with IgG antibodies specific to the antigens of interest as receptors to create immunoFET sensors. The sensors were then exposed to antigens in PBS as well as a number of controls. The conductance was measured for each treatment, and the change in conductance was compared between samples. ImmunoFETs modified with anti-CXCL9 IgG were exposed to 20µg/ml CXCL9. ImmunoFETs modified with anti-IP-10 IgG and anti-RANTES IgG were exposed to IP-10 and RANTES, respectively, at 10ng/ml in PBS. Finally, immunoFETs modified with anti-CXCL9 were exposed to human CXCL9 in murine serum.

Results: We present the successful detection of 20µg/ml CXCL9 in PBS using an immunoFET modified with anti-CXCL9, as well as detection of 10ng/ml IP-10 and RANTES in PBS using immunoFETs modified with anti-IP-10 and anti-RANTES IgGs, respectively. We also present successful detection of human CXCL9 doped in murine serum using an anti-CXCL9 IgG modified immunoFET.

Conclusion: The presented work clearly demonstrates the feasibility of functional immunoFET sensors in physiological conditions. We present successful detection of multiple unlabeled proteins in physiological buffers of varying complexity. These immunoFETs are to be deployed on indwelling medical materials or on surgical implements to provide real-time quantification and monitoring of inflammatory mediators, providing minimally invasive interrogation in the operating room, at the bedside, or in the clinic. The present studies represent the first step in translation of this exciting technology to human clinical applications.
Symptomatic and Radiographic Evaluation of Hiatal Hernia Recurrence Following Laparoscopic Paraesophageal Hernia Repair with Polyester Composite Mesh Reinforcement

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Introduction: Laparoscopic paraesophageal hernia repair (LPEHR) is the preferred treatment for symptomatic paraesophageal hiatal hernia in specialized centers. LPEHR has yielded excellent perioperative outcomes and symptom control; however, it has been associated with high radiographic recurrence rates. Hiatal reinforcement with PTFE mesh prevents hernia recurrence, but is associated with unacceptable mesh related complications. Conversely, bioabsorbable mesh placement has proven safe, but failed to produce long term reductions in hiatal hernia recurrence. The primary objective of this study was to review a single institution experience to evaluate the initial safety and efficacy of LPEHR with crural reinforcement using a polyester composite mesh.

Methods: A retrospective review of patients undergoing LPEHR from 2006-2011 was conducted under an institutional review board approved protocol. All patients who underwent LPEHR with placement of polyester composite mesh were contacted for study enrollment. Long-term follow-up evaluation was performed in person or by telephone questionnaire. Outcomes included barium esophagram, GERD health related quality of life (GERD-HRQL) assessment, and patient satisfaction with their operation. Significant reflux was defined as a GERD-HRQL score > 12.

Results: Between 2006 and 2011, 175 patients underwent LPEHR, and polyester composite mesh was used for hiatal reinforcement in 29 cases. Twenty (70%) patients completed the questionnaires, and 12 (41%) patients returned for a post-operative barium esophagram to assess for hernia recurrence. The median follow-up interval was 29.5 (6-66) months, and esophagrams were performed at a median of 34 (9 - 66) months following LPEHR. There were no mesh related complications within the study group. Eight of the twelve patients (75%) who underwent a radiographic evaluation with barium had evidence of recurrence. The incidence of significant reflux was 15%. There was no significant difference between the median GERD-HRQL scores between those with radiographic recurrence and those without (p = 0.732). Fifteen percent (n=3) of patients reported moderate to severe dysphasia, and esophagram demonstrated a recurrent hiatal hernia in each case. Eighty-one percent of patients polled reported being satisfied with their surgery, and 86% reported that they would, with the benefit of hindsight, have their surgery again.

Conclusions: LPEHR with polyester composite mesh reinforcement provides durable symptomatic relief with high levels of patient satisfaction at intermediate follow-up. No mesh related complications or side effects occurred in this series. While anatomic hiatal hernia recurrence detected by routine post-operative imaging is common, most of these are asymptomatic and do not correlate with patient symptoms or dissatisfaction with the operation.
MicroRNA Profiling of Problematic Melanocytic Lesions


**Introduction:** MicroRNAs (miRs) are a class of small (19-23 nucleotide), noncoding RNAs that inhibit gene expression by binding to target mRNAs and mediating their degradation or blocking protein translation. miRs play a role in the regulation of many cellular processes, including cellular adhesion, angiogenesis, cell cycle control, and apoptosis. Not unexpectedly, many tumors have altered expression of miRs which can contribute significantly to the maintenance of the malignant phenotype. Our group has investigated the profile of miRs in melanocytic proliferations and shown that there is a distinct miR expression pattern in malignant melanoma tumors. However, the malignant potential of some melanocytic lesions is difficult to predict. These borderline (or indeterminate) lesions create a therapeutic dilemma in which undertreatment of lesions, incorrectly thought to be benign, could adversely impact survival. Conversely, overtreatment of lesions thought to be malignant could result in unnecessary morbidity from surgery or adjuvant therapy. We hypothesized that characterization of miR expression in borderline melanocytic proliferations (e.g., atypical Spitz tumors) would lead to the identification of a molecular profile that identifies lesions with high malignant potential, requiring more aggressive therapy.

**Methods:** Based on our group’s previous microarray studies and a search of the current literature, twelve miRs that have been found to be dysregulated in melanoma as compared to benign nevi were selected for analysis. The following miRs were evaluated: let-7a, miR-17-5p, miR-21, miR-22, miR-23b, miR-34a, miR-125b, miR-148b, miR-150, miR-155, miR-200c, and miR-211. RNA extraction was performed on formalin fixed, paraffin embedded tissue samples from the following melanocytic lesions: benign nevi, atypical Spitz tumors, and malignant melanoma. The miR expression profiles of the lesions were evaluated by real-time PCR.

**Results:** Previous microarray analysis of primary melanoma lesions demonstrated that several miRs were upregulated in malignant melanoma tumors as compared to benign nevi by microarray: miR-17-5p, miR-21, miR-107, miR-130a, miR-155, miR-181b, and miR-221. miR-211 was identified as being downregulated in melanoma by microarray. These miRs were further evaluated by real-time PCR in an additional 28 malignant melanoma tumors, which revealed that primary melanomas had an 8.6-fold overexpression of miR-21 and a 7.5-fold overexpression of miR-155 compared with benign nevi (P<0.0001). Expression of the remaining miRs trended as had been predicted by the microarray data, but the results did not reach statistical significance. Additional miRs were hypothesized to be up-regulated (miR-17-5p, miR-21, miR-22, miR-150, miR-155) or down-regulated (let-7a, miR-23b, miR-34a, miR-125b, miR-148b, miR-200c, miR-211) based upon a review of the literature. This group of miRs was then applied to a selected panel of atypical Spitz tumors (n=12) that had presented a diagnostic dilemma to the OSU melanoma multi-disciplinary team. A panel of ten benign nevi was used as a control. Significant, 3.5-fold, overexpression was observed for miR-23b in atypical Spitz tumors (fold expression vs RNU6B = 0.53 ± 0.39) compared to benign nevi (fold expression vs RNU6B = 0.15 ± 0.13, P<0.008). Significant underexpression was observed for miR-125b (3.2-fold) and miR-211 (6.0-fold) in atypical Spitz tumors compared to benign nevi (P<0.002 and P<0.01, respectively). Likewise, benign nevi could be readily identified via their miR profile demonstrating overexpression of miR-125b and miR-211. No significant difference in expression was observed for the remaining miRs in atypical Spitz tumors as compared to benign nevi.

**Conclusions:** MicroRNA expression profiles can be used to characterize problematic melanocytic lesions that are difficult to differentiate as benign or malignant. This profile may assist in determining the malignant potential of problematic pigmented lesions, and provide guidance to clinicians in the selection of surgical and adjuvant therapies.
A Scoring System for Prognosis and Treatment of Malignant Bowel Obstruction

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Introduction: Malignant bowel obstruction is a common result of end-stage abdominal cancer that is a treatment dilemma for many physicians. Little has been reported predicting outcomes or determining the role of surgical intervention. We sought to review our experience with surgical and non-surgical management of malignant bowel obstruction to identify predictors of 30-day mortality and of who would most likely benefit from surgical intervention.

Methods: A chart review of 523 patients treated between 2000 and 2007 with malignant bowel obstruction were evaluated for factors present at admission to determine return to oral intake, 30-day mortality, and overall survival. Propensity score matching was utilized to homogenize patients treated with and without surgery to identify those who would benefit most from operative intervention.

Results: Radiographic evidence of complete obstruction of the small bowel (versus partial obstruction or large bowel obstruction) was predictive of return to oral intake. Hypoalbuminemia and radiographic evidence of ascites or carcinomatosis were all predictive of increased 30-day mortality and overall survival. A nomogram of five identified risk factors correlated with increased 30-day mortality independent of therapy. Patients with large bowel or partial small bowel obstruction benefited most from surgery. A second nomogram was created from four identified risk factors that demonstrated which patients with complete small bowel obstruction might benefit from surgery.

Conclusions: Two nomograms were created that may guide decisions in the care of patients with malignant bowel obstruction. These nomograms are able to predict 30-day mortality and who may benefit from surgery for small bowel obstruction.
Concomitant Dysregulation of miR-151-3p and miR-126 Correlates with Improved Survival in Resected Cholangiocarcinoma

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Introduction: MicroRNAs (miR) are small non-coding genes which become dysregulated in cancer and have been shown to predict survival. The role of miRs in outcomes for cholangiocarcinoma (CCA) has not been reported.

Methods: RNA was extracted from 32 resected CCA along with adjacent uninvolved bile duct epithelium. A miR profile was identified by Nanostring and validated with RT-PCR. Clinicopathologic characteristics and outcomes were captured and compared. Overall survival curves were created using the Kaplan-Meier method and factors compared by log-rank, chi-square or Cox regression analyses.

Results: A miR expression profile identified 41 miRs to be significantly dysregulated compared to normal. The absolute expression was compared to overall survival after excluding patients with perioperative deaths (N=2). One upregulated microRNA (miR-151-3p, p=0.003) and one downregulated microRNA (miR-126, p=0.023) correlated with survival on univariate analysis. Clinical factors along with these miRs were compared by multivariate analysis. Dysregulation of miRs-151-3p and -126 were the only factors that correlated with improved overall survival (12.3 vs 41.5 months, p<0.000 and 15.1 vs 21.9 months, p=0.006, respectively). Eight patients had dysregulation of both miR-151-3p and -126 while the remainder had dysregulation in only one or none. Concomitant dysregulation of both miRs correlated with the best overall survival (58.7 vs 15.1 months; p=0.000; N=8) (figure); clinicopathologic factors in these groups were otherwise similar.

Conclusions: In resected CCA, both miR-151-3p and miR-126 were the only factors related to an overall improved survival. Further analysis of their targets may yield potential therapeutic or prognostic biomarkers.
Proteasome Inhibition Induces Apoptosis of Melanoma Cells

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Introduction: The ubiquitin-proteasome signaling pathway (UPS) plays a critical role in the ordered, temporal degradation of transcription factors, cyclins, and cyclin-dependent kinase inhibitors required for cell cycle progression. Dysregulation in the UPS is linked to the pathogenesis of various human cancers and therefore targeting components of UPS represents a novel therapeutic treatment strategy in this disease setting. Proteasome inhibition results in the stabilization and accumulation of cell regulatory proteins, leading to the activation of anti-proliferative signals, cell cycle disruption, activation of apoptotic pathways, and, ultimately, cell death. Bortezomib is a first generation proteasome inhibitor that has shown activity as a single agent in multiple myeloma, mantle cell lymphoma and non-small cell lung cancer. We have previously shown that bortezomib and IFN-alpha act synergistically to induce apoptosis in melanoma cell lines by activation of caspase 8 through the association of Fas and FADD. In a subsequent phase I trial evaluating bortezomib and IFN-alpha in metastatic melanoma, 50% of accrued patients had stable disease after one cycle of therapy. MLN2238 is a novel antitumor compound that specifically and reversibly inhibits the 20S proteasome. MLN2238 is administered orally and has shown improved pharmacokinetics and pharmacodynamics in comparison to bortezomib in preclinical studies. We therefore hypothesized that the treatment of human melanoma cells with MLN2238 would enhance tumor cell apoptosis.

Methods: Human melanoma tumor cells (A375, FO1, Mel-39, WM1366) were treated for 48h with various doses of MLN2238 (5 nM - 105 nM). Analysis of apoptosis was subsequently performed via Annexin V-propidium iodide staining. Time course experiments were performed at 12, 24 and 48h using the optimal dose of MLN2238. A375 melanoma cells were treated for 48 hours with 35 nM MLN2238 and analyzed for an apoptotic appearance by light microscopy. Caspase activation, PARP cleavage and FADD expression was evaluated by immunoblot analysis following A375 melanoma cell treatment for 48 hours with IFN-alpha (10^4 U/mL) or MLN2238 (15-65 nM). The combination of MLN2238 and interferon (IFN)-alpha, IL-29, sorafenib (a multi-kinase inhibitor), PLX-4720 (a B-raf inhibitor), or MLN4924 (a NEDD-8 inhibitor) on melanoma cell apoptosis was evaluated in a similar fashion. IFN alpha dose = 10^4 U/mL. IL-29 dose = 100 ng/mL. Sorafenib dose = 1 uM. PLX-4720 dose = 100 nM-1 uM.

Results: MLN2238 dose response experiments performed on the A375 cell line identified an optimum in vitro dose of 35 nmol. Treatment of human melanoma cell lines for 48h with MLN2238 resulted in 64.5% cell death on average as compared to 13.4% observed in cells treated with PBS control (p<0.0001). Similar results were obtained with the FO1, Mel-39 and WM1366 cell lines. Time course experiments conducted in the A375 cell line revealed that apoptosis began at 12-24h and reached maximal levels at 48h. Microscopic images of melanoma cells following MLN2238 treatment demonstrated reduced cell size and multiple membrane blebs, characteristic of early apoptosis. Enhanced processing of caspases, cleavage of PARP, and FADD expression was identified by immunoblot analysis following MLN2238 treatment as compared to control conditions. The combination of MLN2238 and IFN-alpha resulted in an average cell death of 62.8%, as compared to 35.2% for MLN2238 alone and 27.8% for IFN-alpha alone. Similarly, the addition of sorafenib to MLN2238 treatment resulted in 82.9% cell death as compared to 64.1% for MLN2238 treatment alone and 12.8% for sorafenib treatment alone. The addition of PLX-4720 to MLN2238 treatment led to less than additive induction of apoptosis: This combination resulted in 56.9% cell death as compared to 25.2% for MLN2238 alone and 40.4% for PLX-4720 treatment alone. MLN2238 therapy had no adverse effects on normal cells including normal human lymphocytes. Studies of MLN2238 therapy in a murine model are ongoing.

Conclusions: MLN2238 is a novel proteasome inhibitor with direct antitumor activity. MLN2238 in combination with IFN-alpha or other targeted agents should be examined further as a potential therapy in melanoma.
Pharmacological Modulation of PTEN Ameliorates the Progression of Pulmonary Hypertension in Heart Failure

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Introduction: Pulmonary hypertension (PH), that occurs secondary to congestive heart failure, leads to vascular remodeling including neointima formation and vascular occlusion. Currently there is a significant gap in the understanding of the mechanisms involved in vascular remodeling, which, if identified, could provide key therapeutic targets. Phosphatase-and-tensin homolog on chromosome 10 (PTEN) has been implicated in arterial remodeling. However, the involvement of PTEN in PH-mediated vascular remodeling remains unclear. The objective of the present study was to determine the role of PTEN in PH and to develop a therapeutic strategy.

Methods: PH was induced in rats by ligating the left anterior descending (LAD) coronary artery. The onset of PH was monitored by echocardiography and confirmed by hemodynamic measurements. Rats were continuously treated with 100-ppm HO-3867, a promoter of PTEN expression, in the feed for 4 weeks. Control groups did not receive HO-3867. The vascular smooth muscle cells (vSMCs) in the lung were collected using laser capture microdissection. The cells and whole lung tissues were analyzed by western blot, RT-PCR, and qRT-PCR.

Results: The HO-3867 treatment group had a significantly higher ejection fraction (EF) compared to control. Pulmonary arterial and RV systolic pressure data showed the development of PH at 4 weeks post-LAD ligation. Phosphorylated PTEN (Ser380/Thr382/383) was markedly depressed in the PH lungs (43.8\% compared to non-PH). Rats treated with HO-3867 showed a significant recovery of PTEN (57.61\%). Focal adhesion kinase (FAK) expression was higher in the PH group compared to the HO-3867-treated group. Similar results were obtained at the mRNA levels of the key proteins in the vSMCs collected from lung.

Conclusions: Deregulation of PTEN is involved in PH-mediated vascular remodeling. The vascular remodeling can be inhibited by targeting PTEN pathway using PTEN-promoting agents such as HO-3867.
Porcine Wet Lab Improves Surgical Skills in Third Year Medical Students

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Introduction: Medical students entering clinical rotations desire to become proficient in surgical techniques and believe their acquisition is important. However, learning these skills in the operating room is challenging. Furthermore, anxiety, low confidence, and poor self-assessment are common during early performance. Medical schools commonly employ small group procedural workshops to improve confidence, participation, and performance. Workshops employing fresh animal tissues are highly rated among medical students and improve basic surgical techniques. Additionally, greater exposure to operative procedures and surgical staff may positively influence students’ desire to pursue surgical careers. We hypothesized that a porcine wet lab for third year medical students would improve procedural skills.

Methods: Two skills labs were conducted sequentially for third year medical students during surgery clerkships in the fall of 2011. Prior to participation, each student was evaluated by a senior resident or attending surgeon on nine basic surgical skills witnessed in the operating room.

During each skills lab, medical students were divided into small groups, and performed the following procedures under direct supervision of a surgical resident or faculty member:

1. Exploratory laparotomy
2. Small bowel resection with hand-sewn anastomosis
3. Splenectomy
4. Partial hepatectomy
5. Cholecystectomy
6. Interrupted abdominal wall closure
7. Running abdominal wall closure
8. Skin closure (stapled and sutured)

Following the skills lab, students were re-evaluated in the operating room by senior residents or attending surgeons using the same evaluation form. Students also provided feedback on their experience.

Fifty-one participants provided pre- and post-test data used in final analysis. Mean values were calculated for each of the nine surgical procedures and compared to corresponding mean values from post-test evaluations (n = 51). A cumulative mean score combining all nine parameters was also compared between pre- and post-tests (n = 51).

Results: Mean scores for each of the nine surgical skills improved significantly after participating in the skills lab (p < .002). Cumulative post-test scores also showed significant improvement compared to cumulative pre-test scores (p = .002). Feedback provided by students revealed overwhelmingly enthusiastic reviews.

Conclusion: Third year medical students demonstrated significant improvement in surgical skills after participation in a porcine wet lab and rated the experience as highly educational. Integration into the current third year surgical curriculum would promote surgical skill proficiency and may elicit interest in surgical careers.