

UNIVERSITY OF WISCONSIN

17th Annual Resident Research Day

Madison
Museum of
Contemporary
Art

May 10
2017



Visiting Professor



Brian Zuckerbraun, MD, FACS

Chief, Division of General/Trauma and Acute Care Surgery

Professor of Surgery

University of Pittsburgh



Brian Zuckerbraun, MD, FACS, is currently the *Henry T. Bahnson Professor of Surgery* at the University of Pittsburgh and *Chief, Division of General and Trauma Surgery* at the University of Pittsburgh Medical Center. He is a graduate of Northwestern University's Honors Program In Medical Education. Dr. Zuckerbraun performed his general surgery residency and research fellowship at the University of Pittsburgh from 1997-2005 and then joined the faculty at the University of Pittsburgh. He works clinically at UPMC and the VA Pittsburgh Healthcare System. Dr. Zuckerbraun has a research lab focusing on tissue protective responses to prevent organ injury from trauma or sepsis and is funded by the National Institutes of Health, the VA, and the Department of Defense. Dr. Zuckerbraun is a member of numerous societies, including the American Society of Clinical Investigation and Association of American Physicians. He has published over 160 original articles, reviews, and chapters. Dr. Zuckerbraun has clinical expertise in multiple areas, including trauma, surgical infections, acute care surgery, and hernia and abdominal wall surgery.

Agenda



Grand Rounds Presentation

Health Sciences Learning Center, Room 1345

AM

7:20

Shock and the Story of an Asphyxiant Gas

Brian Zuckerbraun, MD, FACS, Visiting Professor – University of Pittsburgh

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Resident Research Presentations

Madison Museum of Contemporary Art

10:00

Introduction and Welcome

Jacob Greenberg, MD, EdM

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Barriers to Goal-Concordant Care for Older Patients with Acute Surgical Illness: Communication Patterns Extrinsic to Decision Aids

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Machine Learning to Identify Multigland Disease in Primary Hyperparathyroidism

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Feasibility of an Image-Based Mobile Health Protocol for Postoperative Wound Monitoring

Rebecca Gunter, MD

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A Pediatric Burn Outpatient Short Stay Program Decreases Patient Length of Stay with Equivalent Burn Outcomes

Tiffany Zens, MD

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Residents' Surgical Performance During the Laboratory Years: An Analysis of Rule-Based Errors

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Barriers to Goal-Concordant Care for Older Patients with Acute Surgical Illness: Communication Patterns Extrinsic to Decision Aids

Lauren J Taylor, MD; Sara K Johnson, MD; Michael J Nabozny, MD; Jennifer L Tucholka, BS; Nicole M Steffens, MPH; Kristine L Kwekkeboom, PhD, RN, FAAN; Karen J Brasel MD, MPH, Toby C Campbell, MD, MSCI; Margaret L Schwarze, MD, MPP

Objective

We sought to characterize patterns of communication extrinsic to a decision aid that may impede goal-concordant care.

Background

Decision aids are designed to facilitate difficult clinical decisions by providing better treatment information. However, these interventions may not be sufficient to effectively reveal patient values and promote preference-aligned decisions for seriously ill, older adults.

Methods

We conducted a secondary analysis of 31 decision-making conversations between surgeons and frail, older inpatients with acute surgical problems at a single tertiary care hospital. Conversations occurred before and after surgeons were trained to use a decision aid. We used directed qualitative content analysis to characterize patterns within three communication elements: disclosure of prognosis, elicitation of patient preferences, and integration of preferences into a treatment recommendation.

Results

First, surgeons missed an opportunity to break bad news. By focusing on the acute surgical problem and need to make a treatment decision, surgeons failed to expose the life-limiting nature of the patient's illness. Second, surgeons

asked patients to express preference for a specific treatment without gaining knowledge about the patient's priorities or exploring how patients might value specific health states or disabilities. Third, many surgeons struggled to integrate patients' goals and values to make a treatment recommendation. Instead, they presented options and noted, *"It's your decision."*

Conclusions

A decision aid alone may be insufficient to facilitate a decision that is truly shared. Attention to elements beyond provision of treatment information has potential to improve communication and promote goal-concordant care for seriously ill older patients.

Machine Learning to Identify Multigland Disease in Primary Hyperparathyroidism

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Introduction

Most patients with primary hyperparathyroidism (PHPT) have a single adenoma (SA), but 20-25% of cases will have multigland disease (MGD). Preoperative localization of SAs allows for a minimally invasive surgical approach, but these studies are less accurate or unnecessary in MGD. Therefore, pre-operative identification of MGD could direct the need for imaging and inform operative approach. Machine learning (ML) uses computer algorithms to build predictive models from labeled datasets. The purpose of this study is to use ML methods to predict MGD.

Methods

We reviewed a prospectively managed database of patients undergoing

parathyroidectomy from 2001 to 2016. Adult patients with PHPT who underwent initial, curative resection were included. MGD was defined as > 1 gland removed. Patients with genetic syndromes, a history of lithium use, prior neck surgery, or parathyroid carcinoma were excluded. The ML platform WEKA was utilized to compare different classifiers for predicting SA vs MGD from demographic, clinical, and laboratory features. We selected models for 1. overall accuracy and 2. preferential identification of MGD. 10-fold cross validation was used to evaluate accuracy. A review of imaging was performed on a cohort predicted to have MGD.

Results

2010 patients met inclusion criteria: 1532 patients had single adenoma (SA) (76%) and 478 had MGD (24%). After testing many algorithms, we selected two different models for potential integration as clinical decision-support tools. The best overall accuracy was achieved using

a boosted tree classifier, RandomTree: 94.1% accuracy; 94.1% sensitivity, 83.8% specificity, 94.1% positive predictive value, 0.984 Area Under the Receiver Operating Characteristics curve. To maximize positive predictive value of MGD prediction, a rule-based classifier, JRip, with cost-sensitive learning was used and achieved 100% PPV for MGD. Imaging reviewed from the cohort of 34 patients predicted to have MGD by the cost-sensitive model revealed 39 total studies performed: 28 sestamibi scans and 11 ultrasounds. Only 8 (29%) sestamibi scans and 4 (36%) ultrasounds were correct. All 39 imaging studies were potentially avoidable, with cost savings of over \$420/patient.

Conclusions

ML methods can help distinguish MGD early in the clinical evaluation of PHPT, guiding further workup and surgical planning.

Feasibility of an Image-Based Mobile Health Protocol for Postoperative Wound Monitoring

Rebecca L. Gunter, MD; Sara Fernandes-Taylor, PhD; Shahrose Rahman, BS; Lola Awoyinka, MPH; Kyla M. Bennett, MD; Sharon M. Weber, MD FACS; Caprice C. Greenberg, MD MPH FACS; K. Craig Kent, MD FACS

Objective

To establish the feasibility of a patient-centered mobile health protocol for post-discharge wound monitoring using smartphone technology.

Summary Background Data

Surgical site infection (SSI) is the most common nosocomial infection and the leading cause of readmission among surgical patients. Many SSI develop in the post-discharge period and are inadequately recognized by patients. To address this, we developed a mobile health protocol of remote wound monitoring using smartphone technology. The current study aims to establish its feasibility among patients and providers.

Methods

We enrolled vascular surgery patients during their inpatient stay. They were trained to use our mobile health app, which allowed them to transmit digital images of their surgical wound and answer a survey regarding their recovery. Following hospital discharge, participants completed the app daily for two weeks. Providers on the inpatient team reviewed submissions daily and contacted patients for concerning findings.

Results

Forty participants were enrolled. Forty-five percent of participants submitted data every day for two weeks, with an overall submission rate of 90.2%. Submissions were reviewed within an average of 9.7 hours of submission, with 91.9% of submissions reviewed within 24 hours. We detected 7 wound complications with one false negative. Participant and provider satisfaction was universally high.

Conclusions

Patients and their caregivers are willing to participate in a mobile health program aimed at remote monitoring of postoperative recovery, and they are able to complete it with a high level of fidelity and satisfaction. Preliminary results indicate the ability to detect and intervene on wound complications.

A Pediatric Burn Outpatient Short Stay Program Decreases Patient Length of Stay with Equivalent Burn Outcomes

Tiffany Zens, MD, Amy Yan, BS, Cindy Schmitz, MSN, APNP, Lee Faucher, MD, FACS, Angela Gibson, MD, PhD

Background

Traditionally, small pediatric burns are managed with inpatient admission and daily dressing changes. In 2011, our burn center implemented an outpatient short stay (OSS) program in which small pediatric burns were managed as an outpatient utilizing Mepilex Ag™ dressings changed under moderate sedation every 5-7 days.

Methods

Pediatric burn cases were queried for two time periods: prior to the OSS program (2009-2010) and after the OSS program

(2013-2014). Burns >15% total body surface area (TBSA), children with polytrauma, and children >10 years old were excluded. Independent T-tests and Chi Square tests were conducted to analyze differences in patient demographics, burn management and burn outcomes between these groups.

Results

Two hundred nineteen cases were included in the analysis (77 pre-OSS and 142 post-OSS). There was no difference in patient age ($p=0.872$) or TBSA ($p=0.786$) between the groups. The post-OSS group had shorter inpatient length of stay (LOS) (2.93 days vs. 5.21 days, $p<0.001$) and fewer dressing changes (2.32 vs. 4.71, $p<0.001$). There were no changes in readmission

rates ($p=0.375$) or burns requiring grafting ($p=0.155$). Although not reaching statistical significance, less children in the post-OSS group had infectious complications ($p=0.054$) or required reoperation in a two-year follow-up ($p=0.081$). Patient and family satisfaction with the program was high.

Conclusions

Children treated after the implementation of an OSS burn program at the University of Wisconsin had decreased inpatient LOS and fewer painful burn dressing changes. These patients exhibited equivalent, if not superior burn outcomes.

Residents' Surgical Performance During the Laboratory Years: An Analysis of Rule-based Errors

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Introduction

Nearly one-third of surgical residents will enter into academic development during their surgical residency by dedicating time to a research fellowship for one to three years. Major interest lies in understanding how laboratory residents' surgical skills are affected by minimal clinical exposure during academic development. A widely held concern is that the time away from clinical exposure results in surgical skills decay. This study examines the impact of the academic development years on residents' operative performance. We hypothesize that the use of repeated, annual assessments without feedback may still result in learning.

Materials and Methods

Surgical performance data was collected from laboratory residents (post graduate years [PGY] 2-5) during the summers of 2014, 2015, and 2016. Residents had 15 minutes to complete a shortened, simulated laparoscopic ventral hernia (LVH) repair procedure. Final hernia repair skins from all participants were scored using a previously validated checklist. An analysis of variance (ANOVA) test compared the mean performance scores of repeat participants to first time participants.

Results

95 residents (42% female) participated. 27 (37% female) were in laboratory years over the 3-year span of the study. All participants completed the simulated LVH in the allotted time. Laboratory residents who repeated the procedure for a second time (N= 27, 100%) showed an improvement in performance with a mean score of 14 (SE=1.0) in the first year and 17.2

(SD=0.9) in the second year, ($F(1, 52)=5.6$, $p=.022$). Detailed analysis demonstrated improvement in performance for four grading criteria that correlated with rule-based errors made in the first year. There was no improvement in the cognitive errors made in the first year.

Conclusion

Analysis of longitudinal performance of laboratory residents shows higher scores for repeat participants on rule-based errors. These findings suggest that laboratory residents can learn from rule based mistakes when provided with annual performance-based assessments. This benefit was not seen with cognitive errors and has important implications for assessment as learning experiences.

Analysis of Survival After Resection of Pancreatic Adenocarcinoma: When Does Surgical Volume Matter Most?

Alexander V. Fisher, MD, Ahmed Salem, MD, Glen Levenson, PhD, Daniel E. Abbott, MD, Emily R. Winslow, MD, Sharon M. Weber, MD

University of Wisconsin, Department of Surgery

Introduction

Surgical volume clearly affects survival after pancreatectomy. However, it is unknown whether a subset of patients may benefit most from surgery at high volume centers.

Methods

The National Cancer Data Base was used to identify patients undergoing resection for pancreatic adenocarcinoma between 2004-2011. Patients were grouped based on AJCC pathologic stage. Stage-specific Kaplan-Meier survival curves were compared

across hospital volume quartiles using the log-rank test, and Cox proportional hazards modelling performed.

Results

Of 26,105 patients undergoing resection, 4,077 had stage I disease. The volume effect was most significant for Stage I patients, where median survival was 14.4 months longer comparing highest to lowest volume quartiles, whereas only a 4.2 month difference was observed for Stage II, and 1.7 month for Stage III (Figure 1). In Stage I patients, high volume hospitals had more R0 resections (91.9% vs 87.0%, $p<0.0001$), higher average lymph nodes examined (12.5 vs. 8.8, $p<0.0001$), and fewer patients receiving surgery alone (44.6% vs 50.8%, $p<0.0001$). On Cox modeling for Stage I patients, adjusted for socioeconomic and demographic factors,

R0 resection (HR=0.482 [0.423—0.549]) and high (HR=0.752 [0.670—0.845]) or very high hospital volume (0.705 [0.626—0.794]) were strong predictors of survival, while neoadjuvant therapy was associated with worse survival (HR 1.446 [1.270—1.650], $p<0.0001$).

Conclusions

The survival benefit from high volume hospitals is most pronounced for Stage I disease, resulting in 14.4 months improved median survival. Patients with early stage tumors, while perhaps posing fewer operative challenges, potentially represents a subset with the highest incentives to seek high volume surgical centers.



Abstracts

Trends in the Prevalence of Severe Obesity and Bariatric Surgery Access: A State Level Analysis from 2011-2014

Henkel D, Mora-Pinzon MC, Remington PL, Jolles S, Voils CI, Gould JC, Kothari SN, Funk LM

Introduction

Rates of bariatric surgery in Wisconsin previously have been reported to be similar to the national average (1% of the population with severe obesity). Although bariatric surgery access has anecdotally improved in many parts of the country over the past five years, bariatric programs in Wisconsin continue to experience significant problems with insurance coverage. We sought to describe statewide trends in severe obesity demographics and characterize bariatric surgery volume from 2011-2014.

Methods

Data from the Behavioral Risk Factor Surveillance System (BRFSS), a Centers for Disease Control and Prevention (CDC) annual telephone survey that provides state-level estimates for causes of premature death and morbidity among

adults, were analyzed from 2011-2014 for the state of Wisconsin. Overall prevalence estimates of severe obesity (class II and III) were calculated along with severe obesity prevalence estimates according to age group and ethnicity. Odds ratios comparing the likelihood of being severely obese in 2014 versus 2011 were generated within age categories. Bariatric surgery volume data from the Wisconsin Hospital Association for all hospitals in Wisconsin were used to calculate the annual number of bariatric operations per 100 severely obese adults. A 21-question survey was sent to all bariatric surgeons who were members of the Wisconsin state chapter of the American Society for Metabolic and Bariatric Surgery. Survey questions assessed perspectives on bariatric surgery access, insurance coverage, and referral processes.

Results

The overall prevalence of severe obesity in Wisconsin increased by nearly 30% from

2011-2014 (10.4% to 13.2%; $p=0.037$) (**Table 1**). Severe obesity rates for Black, Hispanic and White adults were 21.7%, 13.9%, and 11.2%, respectively. The odds of severe obesity in 2014 vs. 2011 nearly doubled for adults age 20-39 (OR 1.9, 95% CI 1.3-3.0). The volume of bariatric surgery declined from 1,432 in 2011 to 1,372 in 2014 ($p<0.001$). The number of laparoscopic Roux-en-Y gastric bypasses declined by 60% from 2011-2014 (1,252 to 721; $p<0.001$), while laparoscopic vertical sleeve gastrectomy volume increased five-fold (109 to 644; $p<0.001$). 0.35 per 100 adults with severe obesity underwent bariatric surgery in 2011 vs. 0.26 in 2014. Of the 32 bariatric surgeons who were identified in Wisconsin, 63% completed the survey. Of those, 72% felt that bariatric surgery access had either worsened or remained the same over the last five years.

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Conclusion

Severe obesity has rapidly increased in Wisconsin over the past five years, particularly among young adults. Bariatric surgery volume, on the other hand, has remained largely unchanged and is now substantially below the per capita national average. These observations warrant a comprehensive, population-based public health strategy aimed at improving care and access to evidence-based treatment for patients with severe obesity. Similar analyses in other states may offer insight into the extent of bariatric surgery variability throughout the U.S.

Table 1—Obesity and Bariatric Surgery Trends in Wisconsin (2011-2014)						
Class II and III Obesity						
	2011 % (95% CI)	2012 % (95% CI)	2013 % (95% CI)	2014 % (95% CI)	P value for trend	Likelihood of Severe Obesity in 2014 vs. 2011: Odds Ratio (95% CI)
Age 20-39	7.1 (4.7-9.5)	10.7 (8.1-13.2)	9.4 (7.2-11.6)	12.8 (10.4-15.3)	0.015	1.9 (1.3-3.0)
Age 40-59	13.5 (11.1-15.9)	14.3 (11.9-16.6)	14.6 (12.3-17.0)	14.6 (12.6-16.8)	0.896	1.1 (0.8-1.4)
Age > 60	10.1 (8.4-11.9)	10.8 (8.7-12.9)	10.8 (9.0-12.7)	11.8 (10.0-13.5)	0.644	1.2 (0.9-1.5)
Age >20	10.4 (9.1-11.8)	12.1 (10.7-13.5)	11.8 (10.5-13.1)	13.2 (11.9-14.4)	0.037	N/A
Bariatric Surgery (number of cases)						
Lap RYGB	1,252	1,084	928	721	<0.001	
Lap VSG	109	342	619	644	<0.001	
Other	71	17	8	7	N/A	
Total	1,432	1,443	1,555	1,372	<0.001	
Number of Bariatric Operations per 100 Severely Obese Adults						
	0.35	0.30	0.33	0.26	N/A	

RYGB = Roux-en-Y gastric bypass

VSG = Vertical sleeve gastrectomy

Development of a Mouse Model of Secondary Pulmonary Hypertension: Novel Application of a Model of Ischemic Heart Failure

Philip JL, Tabima DM, Hacker TA, and Chesler NC

Background

Heart failure (HF) is the most common cause of pulmonary hypertension (PH). In patients with HF, elevated pulmonary artery pressures are associated with poor outcomes including increased risk of death and hospitalization as well as increased risk of early post-operative morbidity and mortality following heart transplant. PH and increased pulmonary vascular resistance are a relative contraindication to heart transplantation. However, many of the mechanisms of pulmonary vascular remodeling driving reversible and irreversible PH in the setting of left heart failure (LHF) are unknown. Currently there are few therapies for PH due to LHF beyond optimization of medical therapies for LHF and some limited application of therapies developed for idiopathic pulmonary artery hypertension. We sought to develop a small animal model of PH due to LHF in order to characterize both the

ventricular and vascular biomechanics and pathologic vascular remodeling.

Methods

LHF was created in 6 week old C57/Bl6 mice using a myocardial infarction (MI) model. MI was created via left anterior descending coronary artery (LAD) ligation. Sham animals who underwent thoracotomy without LAD ligation were used as controls. Animals underwent serial echocardiography (echo) examining biventricular function and right heart catheterization at 12 weeks post-surgery measuring in vivo right ventricular (RV) and pulmonary artery (PA) function via pressure-volume measurements.

Results

At 4 weeks post-surgery, MI mice developed left ventricular (LV) systolic dysfunction with decreased ejection fraction and cardiac index which persisted at 12 weeks post-MI. MI mice developed RV hypertrophy with higher RV weight

normalized to body weight compared to sham MI (1.02 ± 0.09 vs 0.80 ± 0.03 , $p=0.024$). MI mice developed significantly higher RV systolic pressure at 12 week post-MI vs Sham (29 ± 1.2 vs 20 ± 1.0 mmHg, $p<0.0001$). MI mice were characterized by decreased RV contractile function as measured by decreased RV end-systolic elastance and significantly increased PA elastance, a marked of pulmonary vascular (PV) resistance and PA stiffening. These resulted significant in RV-PV uncoupling (0.42 ± 0.05 vs 1.11 ± 0.12 , $p<0.005$), a marker of RV dysfunction.

Conclusions

This study utilizes the well-established LAD ligation model of ischemic left heart failure in order to evaluate and characterize the development of secondary pulmonary hypertension due to LHF. These studies will provide improved understanding of the pathophysiology of this deadly disease as well as help to identify novel therapeutic targets.

Prolongation of Transplant Skin Allograft Survival with Dietary Supplementation of Aryl Hydrocarbon Receptor Ligands

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The Aryl Hydrocarbon Receptor (AHR) is a cytosolic transcription factor with numerous endogenous and xenobiotic ligands playing a key role in a number of cellular processes especially immune cell function and development. Differential effects of AHR ligands upon CD4 naïve Tcell development has been described with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inducing regulatory T cells while FICZ promotes Th17 responses. Previous work in our lab has demonstrated prolongation of graft survival in a fully mismatched skin allograft rejection model (Balb/c donor

to C57BL/6 recipient) with intraperitoneal administration of TCDD and early rejection with FICZ treatment. In the present work we demonstrate prolonged graft survival in a minor mismatch allograft rejection model (C57BL/6 Male to Female) with dietary supplementation with the AHR ligand, Indole-3-Carbinol (I3C). 7 week old C57BL/6 female mice were maintained on semi-purified base (SPB) diet deficient of AHR-ligands or SPB diet with I3C supplementation (3ppm) for 3wks prior to transplant and for the duration of the model. AHR-mediated graft survival was correlated to decreases in IFN γ producing Graft Infiltrating Leukocytes (GILs). Animals maintained on SPB diet demonstrated a loss of intestinal Treg, Innate Lymphoid Cells type 3, and $\gamma\delta$ Tcells. However, I3C supplementation led to maintenance of these mucosal immune

cell populations as measured by Flow cytometry. Conversely, maintenance of animals on the AHR-deficient SPB diet led to accelerated graft rejection (Median GST of 46 vs 15.5 days $p < 0.05$). These findings were AHR-dependent as AHR null animals were found to not only reject grafts earlier than WT counterparts but were not responsive to I3C supplementation (Median GST, AHR null on AHR-deficient diet vs I3C diet were 17 vs 15.5 days respectively). These data demonstrate a novel regulatory pathway for prolongation of graft survival via dietary supplementation of naturally occurring AHR ligands. While the underlying mechanism remains unclear, these data suggest a role for activation of the mucosal immune system via AHR stimulation leading to regulation of systemic immune responses.

Novel Fusion Protein Targeting Mitochondrial DNA Improves Pancreatic Islet Functional Potency and Islet Transplantation Outcomes

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Long-term graft-survival is an ongoing challenge in the field of islet transplantation. With the growing demand for transplantable organs, therapies to improve organ quality and reduce incidence of graft dysfunction are of paramount importance. We investigated the protective role of a recombinant DNA repair protein targeted to mitochondria (EndoIII), as a therapeutic agent using a rodent model of pancreatic islet transplantation. We first investigated the

effect of therapy on isolated rat islets cultured with pro-inflammatory cytokines (IL-1 β , IFN γ , and TNF α) for 48 hours and documented a significant reduction in apoptosis by flow cytometry, improved viability by immunofluorescence and conserved functional potency in vitro and in vivo in EndoIII treated islets. We then tested the effect of therapy in systemic inflammation using a rat model of donor brain death (BD) sustained for a 6-hour period. An intravenous bolus of EndoIII (4 mg/kg) was given to sham or BD donor animals at the beginning of each experiment and islets were isolated and cultured for 24 hours. Islets purified from EndoIII treated brain-dead donors showed a significant increase in glucose-stimulated insulin release in vitro when compared to islets from vehicle-treated

counterparts. In addition, donor treatment with EndoIII attenuated the effects of BD and significantly improved functional potency of transplanted islets in vivo. Our data indicate that mitochondrially targeted antioxidant therapy is a novel strategy to protect pancreas and islet quality from the deleterious effects of cytokines in culture and during the cytokine storm associated to donation after brain-death. The potential for rapid translational into clinical practice makes EndoIII an attractive therapeutic option for management of brain-dead donors or as an additive to islets in culture after isolation. Further investigation is warranted to test the effectiveness of this approach in the human islet transplant setting.

Genetic and Pharmacologic Modulation of Autophagy in a Mouse Model of Anal Cancer

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University of Wisconsin, Department of Surgery,
Division of General Surgery

Autophagy is an intracellular, catabolic process that maintains cellular health. Regarding cancer, it is speculated to have a dual role, protective against carcinogenesis while promoting tumor growth. We previously reported the importance of autophagic dysfunction early in anal carcinogenesis. To confirm the role of autophagy in tumor development, we employed pharmacologic modulators of this important pathway *in vivo* as well as an *Atg7* knockout variant of our HPV mouse model.

Mice expressing both HPV oncoproteins E6 and E7 (*K14E6/E7*) were treated with the topical carcinogen DMBA weekly and assessed for tumors over 20 weeks. Concurrently, they were given either chloroquine or BEZ235, to inhibit or induce autophagy, respectively. For this group, time to tumor onset, histologic grade, and

immunofluorescence for LC3 β and p62 were examined. Additionally, mice expressing HPV oncogenes E6 and/or E7 were crossed with *K14CreERTM/Atg7^{fl/f}* transgenic mice to generate all possible gene combinations (E6, E7 and/or *CreERTM*). Half of the mice were treated with tamoxifen to knockout *Atg7* and half of the mice were treated with the carcinogen DMBA. Mice were assessed weekly for tumor development and after 20 weeks anal tissue was fixed and graded histologically. ATG7 protein expression was assessed via immunofluorescence.

All DMBA treated *K14E6/E7* mice developed anal cancer, contrary to zero of the no DMBA treated mice. Chloroquine plus DMBA resulted in a significant decrease in the time to tumor onset compared to *K14E6/E7* treated with DMBA alone, while only 40% of BEZ235 plus DMBA treated mice developed anal cancer. Autophagic induction with DMBA and BEZ235, and autophagic inhibition with chloroquine were confirmed via IF. Consistent with published data regarding the two HPV oncoproteins without the *Atg7* or *Cre* transgenes, rates of tumor incidence in mice treated with DMBA were 91% for *K14E6/E7*

K14CreERTM/Atg7^{fl/f}, 33% for *K14E6/K14CreERTM/Atg7^{fl/f}*, 60% in *K14E7/K14CreERTM/Atg7^{fl/f}*, and 0% in *K14CreERTM/Atg7^{fl/f}*. Interestingly, however, in mice lacking both HPV oncogenes (*K14CreERTM/Atg7^{fl/f}*) treated with DMBA and tamoxifen the tumor incidence was 50% (4/8 mice) compared to 0% (0/11 mice) treated with DMBA alone ($P = 0.008$). Tamoxifen treated mice had significantly lower ATG7 expression compared to controls (mean $\pm$ SD; 0.3 ± 0.2 vs 1.3 ± 0.4 , $P<0.0001$).

The development of tumors after *Atg7* knockout in mice lacking both HPV oncogenes signifies an important potential role for autophagy in anal carcinogenesis. Furthermore, pharmacologic inhibition of autophagy promotes tumor development while induction prevents it. Take together, we demonstrate the important role of autophagy in anal carcinogenesis, both pharmacologically and genetically.

Key Words

Anal cancer, HPV, autophagy, anal dysplasia, chemoprevention, BEZ235

The Role of Lymphocyte Specific Protein-1 in Smooth Muscle Cells After Arterial Injury

Mirnal Chaudhary, Go Urabe, Alex Hayden, Sarah Franco, Lian Wang-Guo, Bo Liu, K Craig Kent

Background

In response to vascular injury, vascular smooth muscle cells (SMCs) switch from a differentiated contractile state to synthetic or dedifferentiated phenotype characterized by downregulation of contractile proteins as well as increased rates of proliferation and migration. This phenotypic switch contributes to the pathophysiology of atherosclerosis, aneurysms, as well as vascular interventions. Experimental data generated by our lab indicate that TGF- β , although generally functions as a differentiation factor, downregulates contractile proteins and stimulates migration. To understand how TGF- β promotes SMC phenotypic switch in injured arteries, we performed an Affymetrix Array analysis and identified Lymphocyte Specific Protein-1 (LSP1) among other upregulated genes. LSP1 has been previously noted to play a role in neutrophil transendothelial cell migration. The role of

LSP1 within SMCs however is unknown. We hypothesize that LSP1 contributes to SMC pathophysiological behavior through changes in cell architecture and migration *in-vivo* and *in-vitro*.

Methods and Results

After carotid artery angioplasty, male Sprague-Dawley rats were sacrificed at 3, 7, and 14 days after injury for immunohistochemistry. Immunofluorescence staining revealed a unique upregulation of LSP1 within the neointima, media, and adventitia at 3 and 7 days, with a decline 14 days after injury. Furthermore, confocal images revealed that the LSP1 positive cells minimally express α -SMA (Pierson's Coefficient, $r=.017$). Additional characterization experiments using immune cell markers CD3 and CD45 show no co-localization with LSP1 positive cells. Previous literature demonstrates the ability of LSP1 to target KSR1/MAPK signaling pathway to the cytoskeleton as a part of cell survival. Co-immunoprecipitation experiments confirm an association of LSP1

with KSR1 and ERK2 within rat A10 cells. Furthermore, ICC experiments also confirm an association of LSP1 with the cytoskeleton. Although the knockdown of LSP1 was viable, knockdown followed by Hydrogen Peroxide (75 μ M) treatment to induced apoptosis was associated with significantly increased cell death as measured by Annexin-V/7-AAD double positivity compared to the apoptotic control. Lastly, we demonstrate a near cessation of phosphoERK2 signaling within the LSP1 knockout cells as a potential explanation for its lethality.

Conclusions

These results demonstrate that LSP1 is increased *in-vivo* after balloon injury, and plays a role as a part of targeting KSR1/ERK2 to the cytoskeleton, as well as ERK activation as a part of cell survival *in-vitro*. Experiments to characterize the identity of these LSP1 cells *in-vivo* are in process, with future *in-vitro* experiments to focus on the role of LSP1 as a part of cell survival and cytoskeletal remodeling.