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PISA2017 - Pathobiology for Investigators, Students, and Academicians: Mechanisms of Carcinogenesis: Combating Cancer Through Understanding Pathobiology

September 25-27, 2017

Location: TBD

2016 PISA

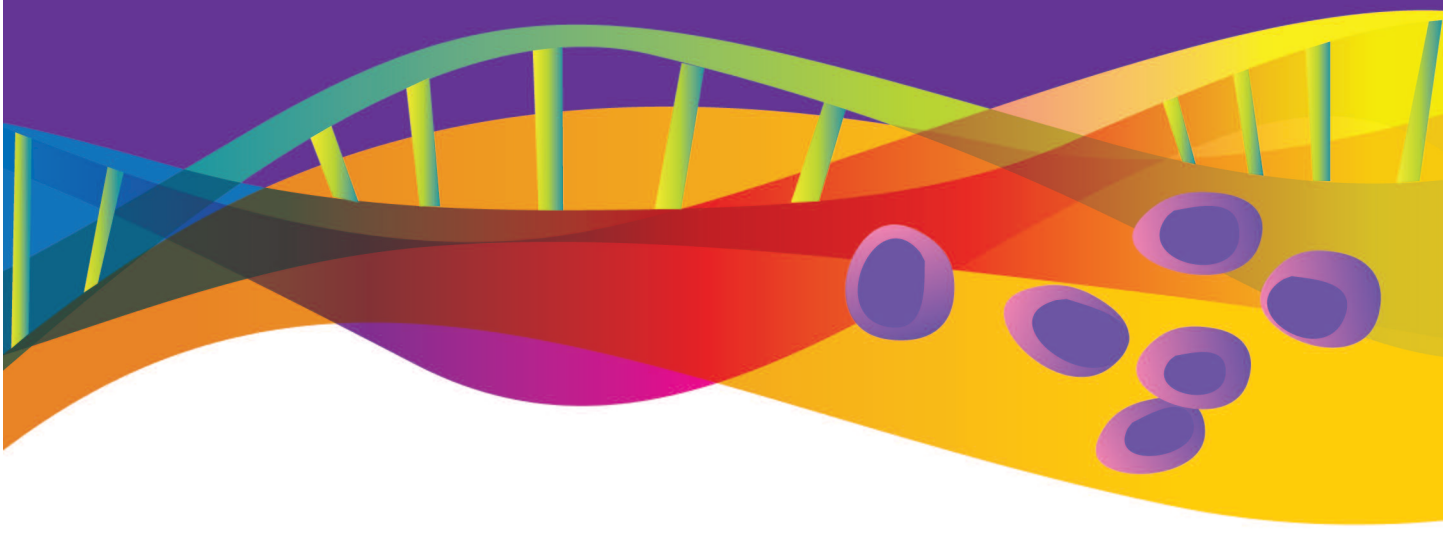
PATHOBIOLOGY FOR INVESTIGATORS, STUDENTS, AND ACADEMICIANS

PISA 2016 Meeting Program

Breakthroughs in Biology: From Underlying Pathogenesis to Translational Medicine

October 20-22, Houston, TX (USA)

www.pisa2016.org



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Histochemical Society

Society for Leukocyte Biology

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Pathobiology for Investigators, Students, and Academicians (PISA) 2016

Breakthroughs in Biology: From Underlying Pathogenesis to Translational Medicine

October 20-22, 2016

Houston, TX (USA)

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Crosswalk — Level 2

The Level 2 Crosswalk connects most buildings on the campus of The Methodist Hospital. Corridors on Levels 1 and 3 provide access to certain buildings and areas as indicated.

MAP LEGEND

- YOU ARE HERE
- CROSSWALK
- EMERGENCY ROOM
- ELEVATORS
- ESCALATORS
- INFORMATION
- RESTROOMS
- PARKING
- VALET PARKING

TRUE NORTH
PLAN NORTH

HELPFUL INFORMATION

General Information
713.441.6000

Transportation Assistance
713.441.4246

Security
713.441.4246

Emergency Department
713.441.1016
Located in Main Building, Level 1

Hospital Address
Houston Methodist Hospital
6565 Fannin St.
Houston, Texas 77030

Valet
Located at:
- Level 1 hospital entries
- Neurosensory/Jones Tower, Level B1 entry

HOUSTON Methodist LEADING MEDICINE

GUIDE TO PISA 2016

Welcome to PISA 2016! ASIP is proud to host this interactive, abstract-driven, focused scientific conference entitled "*Breakthroughs in Biology: From Underlying Pathogenesis to Translational Medicine*" at the Houston Methodist Research Institute (HMRI) and the Houston Marriott Medical Center in Houston, TX. Timely and innovative technologies have vastly impacted our understanding of disease pathogenesis in the last decade. This has come from our improved understanding of the fundamental principles of pathology at cellular and molecular levels. Indeed the fields of immunity/inflammation, vascular and mucosal pathobiology, cell-cell communications, and microbiome/infectious diseases have undergone an unprecedented growth as we reinforce and challenge existing paradigms and discover new ones.

PISA 2016 is aimed at delivering attendees the most exciting and up-to-date concepts in pathogenesis and translational medicine. World acclaimed scientists in these areas will deliver lectures, while experienced members of the program committee will moderate discussion during these sessions to generate a cordial, collegial and contemporary environment for learning and networking. The major lectures will be interspersed with abstract-driven talks. Additionally, poster discussion sessions will be held in the Salons of the Grand Ballroom of the Houston Marriott Medical Center and will build an intimate setting for intellectual exchange and constructive criticism, especially for trainees and junior faculty.

We hope you will take the opportunity to meet and discuss your science with your fellow attendees and invited speakers, not only during the poster-viewing sessions, but during breakfast (Thursday, Friday, Saturday), lunch (Thursday and Friday), the Welcome Reception and Poster Viewing on Thursday evening, and the poster discussion break-outs on Friday afternoon.

THURSDAY, OCTOBER 20, 7:30 AM - 8:25 AM: REGISTER, DISPLAY YOUR POSTER, and ENJOY BREAKFAST

The scientific sessions will be held at the Houston Methodist Research Institute (HMRI) Auditorium at 8:30 AM. Pick up your attendee badge in the HMRI Pre-Meeting Area (2nd Floor) starting at 7:30 AM and enjoy a complimentary continental breakfast.

BEFORE arriving at the HMRI, if you are staying at the Houston Marriott Medical Center, put up your poster in the Marriott Grand Ballroom. It is located on the third floor. If you are arriving locally, please make sure to put up your poster in the Grand Ballroom before arriving at the HMRI. **All posters should be displayed by 8:25 AM.** Posters have been organized into 11 topic categories:

1. Atherosclerosis	A1 & A2
2. Cancer	C1 - C14
3. Cardiomyopathy	CM1 & CM2
4. Genetic Diseases	G1 & G2
5. Immunology	IM1 - IM4
6. Infectious Disease	ID1 - ID7
7. Inflammation	IN1 - IN4
8. Liver Pathobiology	L1 - L16
9. Microbiome	M1 - M6
10. Mucosal Pathobiology	MP1
11. Neuropathology	N1 & N2

After putting up your poster, you can walk across the second floor bridge between the Marriott and Houston Methodist Hospital or go down to the lobby level of the Houston Marriott Medical Center and walk across the street. Follow the signs to the Houston Methodist Research Institute (HMRI) Auditorium (approximately a five-minute walk).

THURSDAY, OCTOBER 20, 8:30 AM: INTRODUCTION TO THE SCIENTIFIC THEMES

Cary D. Austin, Co-chair of the PISA 2016 Steering Committee, will make introductory remarks about the scientific themes of the meeting: Immunity/Inflammation, Mucosal Pathobiology, Cell-Cell Communications, and Microbiome & Disease. **James (Jim) Musser, Chair of Pathology and Genomic Medicine at HMRI** will follow with a "Texas Welcome" that will set the tone for our Keynote Lecture.

THURSDAY, OCTOBER 20, 8:35 AM - 9:30 AM: KEYNOTE LECTURE

Sponsored by the Histochecmical Society

Introduction of the Keynote Speaker will be given by **Stephen Hewitt**, editor-in-chief of the *Journal of Histochemistry & Cytochemistry*. **Tanya Mayadas** will present the Keynote lecture on "Targeting Neutrophil Accumulation as a Therapy for Lupus Nephritis".

THURSDAY, OCTOBER 20, 9:30 AM - 12:20 PM: PLENARY SESSION: THE INFLAMMATORY INTERFACE: THE FORCE AWAKENS

Immediately after the Keynote Lecture, the Plenary Session on The Inflammatory Interface: The Force Awakens will start with an introduction from **Richard (Rick) Mitchell f**ollowed by a lecture from **Lauri Diehl** on Age-Related Macular Degeneration and an abstract-driven short talk by **Ali Alawieh (Abstract IM1).**

After a short (15-minute) break, **William (Bill) Muller** will present a plenary lecture on Leukocyte/ Endothelial Cell Signaling, followed by **David Sullivan** who will give an abstract-driven short talk (**Abstract IN3**). We will end before lunch with a lecture on Host-Pathogen Interactions by **David Walker**.

THURSDAY, OCTOBER 20, 12:20 PM - 1:50 PM: LUNCH AND POSTER VIEWING

Supported by Elsevier, Inc.

Lunch will be held at the Houston Marriott Medical Center in the Grand Ballroom. Take some extra time to familiarize yourself with the posters, engage in conversation with your colleagues, or stroll outside before returning to the HMRI to attend the plenary session on Microbiome and Disease.

THURSDAY, OCTOBER 20, 2:00 PM - 4:35 PM: PLENARY SESSION MICROBIOME AND DISEASE

The Plenary Session on Microbiome and Disease will begin with an introduction by **Cecelia Yates**. **Andrew (Andy) Neish** will speak about Host-Microbiota Interactions at the Epithelial Surface, followed by short abstract-driven talks by **Leon Zheng (Abstract M6) and Deisy Contreras (Abstract ID2)**. **James (Jim) Versalovic** will then give his lecture on Manipulating Gut Microbial Metabolism and Intestinal Inflammation. Finally, **Jonathan (Jon) Braun** will tie the afternoon's session together with his closing lecture on The Search for Correctible Pre-Disease States of the Microbiome.

But wait, there's more! The major poster session and opening reception begins immediately after the final lecture. Meet you back at the Houston Marriott Medical Center in the Grand Ballroom!

THURSDAY, OCTOBER 20, 4:45 PM - 6:30 PM: WELCOME RECEPTION & POSTER SESSION

Take some time to mingle with your colleagues and enjoy a buffet of "small bites" and beverages as you view the posters in the Grand Ballroom of the Houston Marriott Medical Center.

Odd-numbered posters will be manned from 5:00 PM – 5:30 PM. The even-numbered posters will be manned from 5:30 PM – 6:00 PM. This is a great opportunity to exchange ideas, and develop collaborations! The reception ends at 6:30. Leave your poster on its board. It will be moved for you into a

new location for Friday afternoon's Poster Breakout Session. Go out for dinner with your old and new friends and enjoy the many restaurants in the Houston area.

FRIDAY, OCTOBER 21, 7:30 AM - 8:55 AM: ENJOY BREAKFAST

Enjoy a complimentary continental breakfast in the Pre-Meeting Area of the HMRI to start off your day!

FRIDAY, OCTOBER 21, 7:45 AM - 8:55 AM: CAREER DEVELOPMENT WORKSHOP – DANCING WITH JOURNALS: THE MECHANICS OF PUBLISHING YOUR RESEARCH

Supported by The American Association of Immunologists, Inc.

We will start the morning in the HMRI Boardroom with **Kathryn Stockbauer** who will present a workshop on The Mechanics of Publishing Your Research. This session is targeted to young investigators and is sponsored by the ASIP Committee for Career Development and Diversity. Breakfast will be available through 8:55 AM for those who choose not to attend the Workshop.

FRIDAY, OCTOBER 21, 8:55 AM - 9:30 AM: YOUNG SCIENTIST LEADERSHIP AWARD LECTURE

Join us in the HMRI Auditorium where **F. William (Bill) Luscinskas,** Co-chair of the PISA 2016 Steering Committee, will welcome back our attendees and introduce **Christi Kolarcik** who will present the ASIP 2016 Young Scientist Award Lecture entitled "Approaching Amyotrophic Lateral Sclerosis from Cellular, System and Technological Angles".

FRIDAY, OCTOBER 21, 9:30 AM - 12:20 PM: CELL-CELL COMMUNICATIONS

The Plenary Session on Cell-Cell Communications will begin with an introduction by **Christopher (Chris) Moskaluk** followed by a lecture from **Raghu Kalluri** on Exosomes in Pancreatic Cancer Therapy and Diagnosis and an abstract-driven short talk by **Nianxi Zhao (Abstract C10)**.

After a 15-minute break, we will continue with a lecture from **Dihua Yu,** which will cover Exosomes and Extracellular miRNAs in Brain Metastases, followed by **Biju Parekkadan** who will discuss Engineered Systems to Control Cell - Cell Communication.

FRIDAY, OCTOBER 21, 12:20 PM - 1:50 PM: LUNCH

Enjoy lunch with your colleagues in the HMRI Pre-Meeting Area.

FRIDAY, OCTOBER 21, 2:00 PM - 2:50 PM: ROUS-WHIPPLE AWARD LECTURE

The final afternoon oral session will be "lassoed" by **Jim Musser** as he presents The Rous-Whipple Award Lecture on Molecular Triggers Underlying Pandemics Caused by Group A Streptococcus, the Flesh-Eating Pathogen. The break-out poster discussions begin in a half-hour. Meet you at the Houston Marriott Medical Center.

FRIDAY, OCTOBER 21, 3:00 PM - 4:50 PM: POSTER DISCUSSION BREAK-OUTS

The networking highlight of the meeting will occur during the poster discussion break-out sessions. The posters will be set-up in 4 special break-out rooms (see below). The Liver Pathobiology posters will be displayed in **Salons A, B & C**, the Cancer poster will be in **Salon D**, the Microbiome and Infectious Diseases posters will be in **Salon E**, and the Cell Injury, Inflammation, Immunopathology and Disease posters will be in **Salons G & F**. Each break-out group will start with a tour of the posters. **Each author will give a "lightning round" 3-minute presentation of their poster.** After the group has completed the lightning round, the moderator(s) will lead an interactive discussion of the posters. **All posters must be removed after the poster discussion break-outs.**

BREAK-OUT GROUPS

Liver Pathobiology

- Posters: L1 - L16

Cancer

- Posters: C1 - C14

Microbiome and Infectious Diseases

- Posters: ID1 - ID7, M1 - M6

Cell Injury, Inflammation, Immunopathology and Disease

- Posters: A1 & A2, CM1 & CM2, IM1 - IM4, MP1, IN1 - IN4, N1& N2, G1 & G2

FRIDAY, OCTOBER 21, 5:00 PM - 6:00 PM: SCIENTIFIC INTEREST GROUP (SIG) MEETING

At the conclusion of the poster discussion break-outs, various Scientific Interest Groups (SIG) will have a chance to meet. All ASIP members belong to at least one SIG, so be our guest at this interactive, informal meeting where you will meet the ASIP Leadership, SIG Chairs, and partake in lively and informative discussions concerning your scientific area of interest. If you do not remember which SIGs you have joined, no need to worry, stop by the Registration Desk and we will have that information for you!

SATURDAY, OCTOBER 22, 7:30 AM - 8:25 AM: ENJOY BREAKFAST

Enjoy a complimentary continental breakfast in the HMRI Pre-Meeting Area.

SATURDAY, OCTOBER 22, 8:25 AM - 10:40 AM: PLENARY SESSION ON MUCOSAL PATHOBIOLOGY

The Plenary Session on Mucosal Pathobiology will begin with an introduction by **Bill Luscinskas**, followed by a lecture from **Sean Colgan** on Metabolic Shifts and Innate Immune Responses in the Gut and two abstract-driven short talks from **Michelle Reed (abstract MP1)** and **Tirthadipa Pradhan-Sundd (Abstract L14)**. After a 15-minute break, we will return for the final lecture of PISA 2016, which will be presented by **Nicholas Lukacs** on Microbiome, Immune Function, and Pulmonary Disease.

SATURDAY, OCTOBER 22, 10:40 AM - 11:10 AM: ASIP BUSINESS MEETING AND AWARDS PRESENTATION

The ASIP Business Meeting and Awards Presentation will be chaired by **ASIP President George Michalopoulos.** The ASIP Leadership will briefly present reports that reflect the state of the Society. Dr. Michalopoulos will then present awards to honor recipients of the ASIP 2017 Rous-Whipple Award, ASIP 2017 Robbins Distinguished Educator Award, ASIP 2016 Young Scientist Leadership Award, Junior Faculty Travel Awards, Trainee Travel Awards, Promoting Diversity in Science Travel Award, and the GALL Travel Award for Excellence in Cardiovascular Research. **Awardees must be present to receive their award!**

SATURDAY, OCTOBER 22, 11:15 AM - 12:00 PM: MEETING OF THE SIG CHAIRS

A special meeting of the Scientific Interest Group (SIG) Chairs will be held in the HMRI Boardroom (next to the auditorium). If you are SIG Chair, please attend.

At the conclusion of the meeting, please complete a PISA 2016 Meeting Evaluation. Your input will be of value as we plan the scientific program and schedule for PISA 2017 and the next ASIP Annual Meeting at EB17. If you complete your survey onsite, you will receive a "special" treat! The survey can also be completed online by navigating to the link below.

(www.pisa2016.org/survey)

Plans are underway for PISA 2017, which is scheduled for September 25-27, 2017. Please stay tuned for details. We look forward to seeing you there!

PISA 2016 MEETING PROGRAM

All sessions are being held at the Houston Methodist Research Institute (HMRI), unless otherwise noted.

THURSDAY, OCTOBER 20, 2016

7:30 AM - 8:25 AM	**Registration/Breakfast**
8:30 AM - 8:35 AM	**Welcome** *Cary D. Austin, MD, PhD and James M. Musser, MD, PhD*
8:35 AM - 8:40 AM	Introduction of Keynote Speaker - *Stephen Hewitt, MD, PhD*
8:40 AM - 9:30 AM	**Keynote Lecture** - Journal of Histochemistry & Cytochemistry Lecture: Targeting Neutrophil Accumulation as a Therapy for Lupus Nephritis *Tanya Mayadas, PhD, Brigham and Women's Hospital and Harvard Medical School* *Sponsored by the Histochecmical Society*
9:30 AM - 9:35 AM	**The Inflammatory Interface: The Force Awakens** *Introduction - Richard N. Mitchell* *Chairs: Cary D. Austin, Richard N. Mitchell, William A. Muller*
9:35 AM - 10:15 AM	**Age-Related Macular Degeneration: Evaluating the Role of Complement as a Therapeutic Opportunity in Geographic Atrophy** *Lauri Diehl, DVM, PhD, Genentech, Inc.*
10:15 AM - 10:30 AM	**Abstract-Driven Short Talk - A Novel Injury Site-Specific Complement Inhibitor Reduces Neuronal Loss and Prevents Chronic Inflammation After Murine Stroke (IM1)** *Ali Alawieh, BS, Medical University of South Carolina*
10:30 AM - 10:45 AM	Break
10:45 AM - 11:25 AM	**Leukocyte/Endothelial Cell Signaling During Transmigration** *William A. Muller, MD, PhD, Northwestern University Feinberg School of Medicine*
11:25 AM - 11:40 AM	**Abstract-Driven Short Talk - The Role of IQGAP1 in Transendothelial Migration: From In Vitro Identification to In Vivo Validation (IN3)** *David Sullivan, PhD, Northwestern University Feinberg School of Medicine*
11:40 AM - 12:20 PM	**Host-Pathogen Interactions** *David Walker, MD, University of Texas Medical Branch - Galveston*
12:20 PM - 1:50 PM	Lunch/Poster Viewing **Houston Marriott Medical Center Grand Ballroom**
2:00 PM - 2:05 PM	**Microbiome and Disease** Introduction - *Cecelia Yates, PhD* *Chairs: James M. Musser, Cecelia C. Yates*

2:05 PM - 2:45 PM **Host-Microbiota Interactions at the Epithelial Surface**
Andrew Neish, MD, Emory School of Medicine

2:45 PM - 3:00 PM **Abstract-Driven Short Talk - Microbial-Derived Short-Chain Fatty Acids Induces Host Interleukin-10 Receptor and Augments Epithelial Barrier Function (M6)**
Leon Zheng, BS, University of Colorado

3:00 PM - 3:15 PM **Abstract-Driven Short Talk - Dysregulation of Long Non-Coding RNA (lncRNA) Genes and Predicted lncRNA-protein Interactions during Zika Virus Infection (ID2)**
Deisy Contreras, BS, Cedars-Sinai Medical Center

3:15 PM - 3:55 PM **Manipulating Gut Microbial Metabolism and Intestinal Inflammation**
James Versalovic, MD, PhD, Texas Children's Hospital and Baylor College of Medicine

3:55 PM - 4:35 PM **The Search for Correctible Pre-Disease States of the Microbiome**
Jonathan Braun, MD, PhD, UCLA Geffen School of Medicine

4:45 PM - 6:30 PM **Welcome Reception/Poster Viewing**
Houston Marriott Medical Center Grand Ballroom

Odd-Numbered Posters	**Even-Numbered Posters**
Presents: 5:00 PM - 5:30 PM	Presents: 5:30 PM - 6:00 PM

Poster Topic Categories:

1.	Atherosclerosis	A1 & A2
2.	Cancer	C1 - C14
3.	Cardiomyopathy	CM1 & CM2
4.	Genetic Diseases	G1 & G2
5.	Immunology	IM1 - IM4
6.	Infectious Disease	ID1 - ID7
7.	Inflammation	IN1 - IN4
8.	Liver Pathobiology	L1 - L16
9.	Microbiome	M1 - M6
10.	Mucosal Pathobiology	MP1
11.	Neuropathology	N1 & N2

FRIDAY, OCTOBER 21, 2016

7:30 AM - 8:55 AM **Registration/Breakfast**

7:45 AM - 8:55 AM **Career Development Workshop - Dancing with Journals: The Mechanics of Publishing Your Research**
Kathryn Stockbauer, PhD, Manager, Academic Development, Houston Methodist Hospital
Sponsored by the ASIP Committee for Career Development and Diversity and The American Association of Immunologists, Inc.

8:55 AM - 9:00 AM	**Welcome** *F. William Luscinskas, PhD*
9:00 AM - 9:30 AM	**ASIP Young Scientist Leadership Award Lecture: Approaching Amyotrophic Lateral Sclerosis from Cellular, System and Technological Angles** *Christi Kolarcik, PhD, University of Pittsburgh*
9:30 AM - 9:35 AM	**Cell-Cell Communications** Introduction - *Christopher Moskaluk, MD, PhD* *Chairs: Satdarshan Paul Monga, Christopher A. Moskaluk, William Stetler-Stevenson*
9:35 AM - 10:15 AM	**Exosomes in Pancreatic Cancer Therapy and Diagnosis** *Raghu Kalluri, MD, PhD, MD Anderson Cancer Center*
10:15 AM - 10:30 AM	**Abstract-Driven Short Talk - High-Payload Aptamer-Drug Conjugates for Targeted Pancreatic Cancer Therapy (C10)** *Nianxi Zhao, PhD, Houston Methodist Research Institute*
10:30 AM - 10:45 AM	Break
10:45 AM - 11:25 AM	**Exosomes and Extracellular miRNAs in Brain Metastases** *Dihua Yu, MD, PhD, MD Anderson Cancer Center*
11:40 AM - 12:20 PM	**Engineered Systems to Control Cell - Cell Communication** *Biju Parekkadan, PhD, Harvard Medical School*
12:20 PM - 1:50 PM	Lunch (HMRI)
2:00 PM - 2:50 PM	**ASIP Rous-Whipple Award Lecture: Molecular Triggers Underlying Pandemics Caused by Group A Streptococcus, the Flesh-Eating Pathogen** *James M. Musser, MD, PhD, Houston Methodist Hospital*
3:00 PM - 4:50 PM	**Poster Discussion Breakouts** **Houston Marriott Medical Center** **Break-Out Groups**: **Liver Pathobiology** • Posters: L1 - L16 **Cancer** • Posters: C1 - C14 **Microbiome and Infectious Diseases** • Posters: ID1 - ID7, M1 - M6 **Cell Injury, Inflammation, Immunopathology and Disease** • Posters: A1 & A2, CM1 & CM2, IM1 - IM4, MP1, IN2 - IN4, N1& N2, G1 & G2
5:00 PM - 6:00 PM	**ASIP Scientific Interest Group Meetings** **Houston Marriott Medical Center**

SATURDAY, OCTOBER 22, 2016

7:30 AM - 8:25 AM	**Registration/Breakfast**
8:25 AM - 8:30 AM	**Welcome** *F. William Luscinskas, PhD*
8:30 AM - 8:35 AM	**Mucosal Pathobiology** ***Introduction*** *- F. William Luscinskas* *Chairs: F. William Luscinskas, Asma Nusrat*
8:35 AM - 9:15 AM	**Metabolic Shifts and Innate Immune Responses in the Gut** *Sean Colgan, PhD, University of Colorado - Denver*
9:15 AM - 9:30 AM	**Abstract-Driven Short Talk - Intestinal Epithelial Expression of CD47 Facilitates Cell Proliferation, Migration, and Mucosal Wound Closure In Vivo (MP1)** *Michelle Reed, PhD, University of Michigan, Ann Arbor*
9:30 AM - 9:45 AM	**Abstract-Driven Short Talk - Simultaneous ablation of ß and γ-Catenin Contributes to Loss of Blood Bile Barrier and Progressive Familial Intrahepatic cholestasis Disease Like Phenotype (L14)** *Tirthadipa Pradhan-Sundd, PhD, University of Pittsburgh*
9:45 AM - 10:00 AM	Break
10:00 AM - 10:40 AM	**Microbiome, Immune Function, and Pulmonary Disease** *Nicholas Lukacs, PhD, University of Michigan*
10:40 AM - 11:10 AM	**Awards Presentations and Business Meeting** Houston Methodist Research Institute Auditorium
11:15 AM - 12:00 PM	**Meeting of Scientific Interest Group (SIG) Chairs** Houston Methodist Research Institute Boardroom

At the conclusion of the meeting, please complete a PISA 2016 Meeting Evaluation. Your input will be of value as we plan the scientific program and schedule for PISA 2017 and the next ASIP Annual Meeting at EB17. If you complete your survey onsite, you will receive a "special" treat! The survey can also be completed online by navigating to the link below.

(**www.pisa2016.org/survey**)

ABSTRACTS

PISA 2016: Breakthroughs in Biology: From Underlying Pathogenesis to Translational Medicine

October 20-22, 2016

Houston Methodist Research Institute, Houston, TX

Abstracts are provided for the convenience of the attendees in planning their poster viewing. They should not be distributed or referenced without the express permission of the authors.

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ATHEROSCLEROSIS	A1-2	INFLAMMATION	IN1-IN4
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GENETIC DISEASES	G1-G2	MUCOSAL PATHOBIOLOGY	MP1
INFECTIOUS DISEASES	ID1-ID7	NEUROPATHOLOGY	N1-N2
IMMUNOLOGY	IM1-IM4		

ATHEROSCLEROSIS

A1 Wnt5a Influences Scavenger Receptor Expression and the Accumulation of Lipid in THP-1 Macrophages

I. Ackers, C. Szymanski, L. Consitt, R. Malgor
Ohio University, Athens, Ohio, USA

Objective: Atherosclerosis is a relatively silent disease consisting of few clinical symptoms with life threatening myocardial infarction and stroke being the initial clinical manifestation. To combat this silent disease there is a critical need to advance our understanding of the pathogenesis of atherosclerosis. Wnt5a, a highly studied member of the Wnt family, has been implicated in various inflammatory diseases including atherosclerosis. We evaluated expression of scavenger receptors (SR) and non-canonical Wnt5a receptors: Frizzled 5 (Fz5) and Receptor tyrosine kinase-like orphan receptor 2 (Ror2) in macrophages/foam cells in atherosclerosis. We hypothesize that Wnt5a contributes to the formation of foam cells through non-canonical Wnt signaling.
Approach and Results: To address this hypothesis, we utilized *in vitro* culture of THP-1 cells (a monocyte cell line) and human atherosclerotic tissue. Western blot shows increased expression of SRB2 (CD36) and decreases in lipid efflux receptor ABCA1 after 1 hour of treatment with recombinant human Wnt5a. Additionally, with LipidTOX staining we found that Wnt5a promoted lipid accumulation in THP-1 cells. THP-1 cells were also found to express Fz5 and Ror2 and expression was influenced by rWnt5a and oxLDL. We also report significantly higher expression of Wnt5a, Fz5, and Ror2, and SR in human advanced lesions compared to less advanced lesions. Importantly, SR expression was coincident with Wnt5a and its receptors in human tissue.
Conclusions: Lipid accumulation within the vessel intima is a major risk factor for development of vulnerable plaques. Our results outline a mechanism by which non-canonical Wnt5a signaling promotes the development of atherosclerosis through dysregulation of lipid handling in macrophages and subsequent formation of foam cells. Our future plan is to evaluate inhibition of Wnt5a signaling as a potential therapeutic target in atherosclerosis through both *in vivo* and *ex vivo* studies.

A2 Functional and Histologic Assessment of Targeted LFA-1 Expression Using ^{111}In-DANBIRT in the Inflammatory Atherosclerotic Plaque

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Objective: Demonstrate the anatomical and functional biodistribution of ^{111}In-DANBIRT using 3D blocking autoradiography, contrasting the histologic and immunohistochemical (IHC) analysis of atherosclerotic vascular lesion development, demonstrating increased expression of LFA-1 by leukocytes within atherosclerotic plaques in mice exposed to a high fat diet.
Methods: ApoE$^{-/-}$ mice were fed either normal or high fat chow (n=8 per group) for 8 weeks; animals were euthanized post-dietary exposure. Hearts were harvested, sectioned and frozen; aortic sub-leaflet OCT frozen cryosections were stained using Oil Red O to assess lipid accumulation in vascular lesion areas in relationship to percentage of affected vessel wall area; immunohistochemical analysis was performed using FITC conjugated anti-mouse CD11a (LFA-1) intraplaque expression. 3D autoradiography was performed in blocked (pre-administered with 1,000X IV dose of cold DANBIRT) and unblocked C57BL6 male mice (n=3 per group) 24 hours post injection of 100µCi of ^{111}In-DANBIRT. Image processing of autoradiography images was performed to allow and analysis by region of interest (ROI) determination in relationship to concentration per volume; values were decay corrected and normalized to muscle uptake; percentage injected dose per gram of tissue (%ID/gr) was calculated from ROI quantification.
Results: ApoE$^{-/-}$ mice on a high fat diet exhibit increased percentage of atherosclerotic lesion to vessel wall area in comparison to normal chow fed mice in Oil Red O stained slides. Epi fluorescent microscopy revealed distinctive intraplaque accumulation of CD11a (LFA-1) expressing leukocytes in vascular atherosclerotic lesions. 3D autoradiography revealed high uptake in unblocked ($p<0.05$) immune-competent lymphoid (thymus, spleen) and cardiovascular tissues (heart, thoracic aorta, right carotid), directly illustrating high specificity where LFA-1 expressing resident and circulating leukocytes are targeted in vivo using ^{111}In-DANBIRT. Data were subject to statistical analysis with two-tailed t-test and/or two-way ANOVA using Graphpad 7.0 for Mac.
Conclusions: 3D blocking autoradiography shows the biodistribution of ^{111}In-DANBIRT in cardiovascular (heart, aortic arch, descending aorta, carotids) and immune tissues in unblocked mice indicating specificity for LFA-1 expression. Atherosclerotic plaque development is evidenced by histologic analysis, which shows high intraplaque lipid accumulation evidencing early stage development of unstable lesions in high fat diet fed mice. IHC fluorescent microscopy showed intraplaque expression of CD11a that correlates with the targeting of LFA-1 in the inflammatory development of the vascular atherosclerotic plaque in our disease model. Findings show a strong relationship to previous longitudinal molecular imaging studies performed with radiolabeled DANBIRT in our laboratory.

^{111}In-DANBIRT continues to endure as a promising radioligand probe for in vivo molecular imaging as a diagnostic tool capable of assessing immune modulation of inflammation in cardiovascular injury models.
Research Support: Data was generated in the Keck-UNM Small-Animal Imaging Resource Shared Resource Center supported by the University of New Mexico Health Sciences Center, the UNM Comprehensive Cancer Center, UNM College of Pharmacy and the Anesthesiology Pain Research Laboratory. Grant numbers: NIH P30CA118100-06 & ES0014639.

CANCER

C1 Alcohol Abuse Promotes Breast Cancer Development and Progression via Sumoylation Status of StarD10

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Background: Epidemiological studies demonstrated that ethanol administration promotes breast cancer development and metastasis. Human breast cancer cells or mammary epithelial cells with a high expression of ErbB2 exhibited an enhanced response to ethanol-stimulated cell invasion *in vitro*. In addition, ethanol stimulates cell proliferation and triggers different intracellular signaling modulating the phosphorylation status of key proteins as JNK, P38 and ERK. Sumoylation is a post-translational modification (PTM) involved in various cellular processes and it has been found to be deregulated in metastatic breast cancer. StarD10 is a member of the start protein family well known to be regulated by PTM; it's a lipid transfer protein with selective binding site to phosphatidylcholine and phosphatidylethanolamine. It was found to be co-expressed with ErbB2 in many breast carcinoma cell lines, suggesting a selective growth advantage for tumor expressing both proteins. The aim of this study was to investigate the role of sumoylated StarD10 in breast cancer and its interaction with ErbB2 as consequence of ethanol administration.
Methods: The experiments were performed using MCF-7, MDA-MB231 human carcinoma cell lines treated with ethanol 100mM. Protein and mRNA levels were analyzed by Western blotting/Co-immunoprecipitation (Co-IP) and RT-PCR, respectively. SUMO-binding columns were used to purity sumoylated protein for Mass Spectrometry analysis.
Results: We found that ethanol treatment increases StarD10 and ErbB2 at both protein and mRNA levels in MCF-7 cells. Recombinant proteins were use to demonstrate the direct interaction between StarD10 and ErbB2 and we confirmed this finding by Co-IP. In addition, ethanol treatment resulted in increased StarD10 and ErB2 interaction. We have previously established that StarD10 was sumoylated in binge mouse liver. Interestingly, we found that overexpression of Stard10 induces cell growth and migration suggesting a potential role of StarD10 in induced-ethanol breast cancer development and/or progression. To obtain additional information on sumoylation sites of StarD10 we used three different prediction software that generated an interesting unconventional non-covalent sumoylation binding-site (aa 130-133).
Conclusion: The ability of Stard10 to cooperate with ErbB receptors may be independent of its lipid binding function. This novel finding could support the hypothesis that sumoylation regulates the interaction between Erbb2 and StarD10 and their synergistic action in the neoplastic progression of the breast cancer. This is the first report demonstrating that the protein StarD10 is also sumoylated in breast cancer cell lines.

C2 **Apigenin Inhibits TNFα / IL-1α**-induced CCL2 Release Through IKBK-epsilon Signaling in MDA-MB-231 Human Triple-negative Breast Cancer Cells.

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Mortality associated with breast cancer is primarily attributable to metastasis, of which TNFα plays a central orchestrating role. Solid breast cancers are circumscribed in a milieu of TNFα secreting cells, which act on tumor cell TNFα receptors evoking a sharp rise in the release of chemotactic proteins (e.g. MCP-1 /CCL2). Subsequently, CCL2 directs inward infiltration of tumor-associated macrophages (TAMs) and other chemokines enabling migration of tumor-associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs), T-regulatory cells (Tregs), T helper IL-17-producing cells (Th17s), metastasis-associated macrophages (MAMs) and cancer-associated fibroblasts (CAFs).
In the current study, we investigated the potential of apigenin, a known anti-inflammatory flavonoid, to downregulate the first step in this process – being the TNFα mediated release of chemokines from human triple-negative breast cancer cells (MDA-MB-231 cells). The data show TNFα to evoke a sharp rise in the release of several proteins: granulocyte-macrophage colony-stimulating factor (GM-CSF), CCL2, IL-1α and IL-6, all effectively attenuated by apigenin. The data also demonstrated the signaling pattern to be associated with cytokine attenuation by apigenin is primarily IKBKe (Inhibitor of kappa light polypeptide gene enhancer in B-Cells, Kinase Epsilon), validated by both (total) mRNA and protein expression.
In summary, these findings suggest that TNFα and IL-1 α are equally capable of evoking the elevated release of CCL2 from breast cancer cells, both attenuated by apigenin - through downregulating IKBKe transcription. Future research will be required to evaluate the capacity of anti-inflammatory nutraceuticals such as apigenin to prevent aggressive metastatic invasion and tumor immune evasion.
This research was supported by the National Institute of Minority Health and Health Disparities of the National Institutes of Health through Grant Number G12 MD007582 and Grant Number P20 MD006738.

C3 Expression of an Aberrant DNA Hypermethylation Gene Expression Signature is Associated With Aggressive Breast Cancer Molecular Subtypes

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Breast cancer is a diverse collection of diseases that can be classified in various ways. We have evaluated the expression of gene expression signatures that are associated with aberrant DNA hypermethylation-dependent gene silencing in a cohort of 855 breast cancers (including 596 ER+ and 256 ER- cancers), including 243 (28%) luminal A, 162 (19%) luminal B, 144 (17%) HER2-enriched, 140 (16%) basal-like, 90 (11%) claudin-low, and 76 (9%) normal-like breast cancers (Harrell et al., 2012, *Breast Cancer Res. Treat.* 132:523-535). The aberrant DNA hypermethylation signature is based upon the expression status of nine methylation-sensitive genes: *CEACAM6*, *CDH1*, *CST6*, *ESR1*, *GNA11*, *MUC1*, *MYB*, *TFF3*, and *SCNN1A* (Sandhu et al., 2012, *Int. J. Oncol.* 41:721-732). 175/855 (20%) breast cancers express the hypermethylator signature, and 681/855 (80%) breast cancers did not. This is consistent with our previous studies that indicated that ~23% of all breast cancers express the hypermethylator gene expression signature (Roll et al., 2013, *Exp. Mol. Pathol.* 95:276-287). Expression of the hypermethylator signature confers a relapse-free survival disadvantage in Kaplan-Meier analyses when all stages and all molecular subtypes of breast cancer are considered (P=0.008), and when stage I/II breast cancers are considered (P=0.001). Compared to non-hypermethylator breast cancers, the breast cancers expressing the hypermethylator signature progress to metastatic disease more frequently (49% versus 43%), and with a shorter disease-free interval (22.4 months versus 34.3 months). Hypermethylator breast cancers metastasize to lung (38%), bone (29%) liver (17%) and brain (16%), compared to non-hypermethylators that metastasize to bone (53%), liver (23%), lung (16%), and brain (8%).These results suggest that breast cancers that express the hypermethylator signature are more aggressive than breast cancers that do not express this signature. Analysis of the breast cancer molecular subtypes associated with the aberrant DNA hypermethylation signature revealed an enrichment for aggressive disease: 85/174 (49%) basal-like, 61/174 (35%) claudin-low, 15/174 (9%) HER2-enriched, 2/174 (1%) luminal A, 6/174 (3%) luminal B, and 5/174 (3%) normal-like. Non-hypermethylator breast cancers are enriched for molecular subtypes associated with better outcomes: 241/681 (36%) luminal A, 156/681 (23%) luminal B, 129/681 (19%) HER2-enriched, 55/681 (8%) basal-like, 29/681 (4%) claudin-low, and 71/681 (10%) normal-like. Most basal-like (85/140, 61%) and claudin-low breast cancers (61/90, 68%) express the aberrant DNA

hypermethylation signature, whereas most luminal A (241/243, 99%), luminal B (156/162, 96%), HER2-enriched (129/144, 90%), and normal-like breast cancers (71/76, 93%) do not express this signature. Expression of the aberrant DNA hypermethylation signature appears to associate with ER- breast cancers. Based upon immunostaining results 138/172 (80%) hypermethylator breast cancers are ER-, and 562/680 (83%) non-hypermethylator breast cancers are ER+ (Fischer's Exact Probability Test: $P<0.0001$). Kaplan-Meier analysis revealed no significant difference in relapse-free survival between hypermethylators and non-hypermethylators when basal-like and claudin-low breast cancers were considered (all stages, $P=0.4529$; stage I/II, $P=0.9476$). These results suggest strongly that patients with breast cancers expressing the aberrant DNA hypermethylation signature (i) are more aggressive than those that do not, (ii) do not respond better to typical chemotherapy regimens, and (iii) may benefit from epigenetic therapy targeting aberrant DNA hypermethylation.

C4 FOXO1/Sprouty-2 Pathway Inhibits Endothelial Cell Tumor Growth

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Endothelial cell tumors (as known as vascular tumors) are neoplasms of endothelial cell origin and have a wide spectrum of clinical presentations, ranging from benign infantile hemangiomas in children to low-grade malignant hemangioendotheliomas and highly aggressive angiosarcomas in adults. To date, the molecular basis of vascular tumor pathogenesis is poorly understood and standard therapy for these tumors has limited clinical efficacy.

Forkhead box protein O1 (FOXO1) is a transcription factor with tumor suppressor function and is dysregulated in human cancer. In this study, we showed that FOXO1 suppressed vascular tumor growth, and mechanistically, the inhibitory effects of FOXO1 were mediated by Sprouty2. FOXO1 expression was reduced in a variety of human vascular tumors examined (infantile hemangioma, hemangioendothelioma and angiosarcoma) as compared with normal blood vessels as determined by western blotting and immunohistochemical stains.

Knockdown of FOXO1 gene expression with short hairpin RNA resulted in increased vascular tumor cell migration and proliferation *in vitro* and *in vivo* animal models. Conversely, over-expression of constitutively active FOXO1 in these cells suppressed cell growth. We observed that FOXO1 interacted with Sprouty2 promoter *in situ* in chromatin immunoprecipitation assay and increased Sprouty2 gene expression in tumor cells. Similar to FOXO1, Sprouty2 expression was reduced in vascular tumors. Over-expression of Sprouty2 decreased tumor cell growth and migration. Conversely, knockdown of Sprouty2 increased tumor growth *in vitro* and *in vivo*. Knockdown of Sprouty2 in cells with over-expression of constitutively active FOXO1 resulted in reduced tumor growth and "rescued" the FOXO1 phenotype, indicating that Sprouty2 is an important mediator of the biological effects of FOXO1.

Microarray gene expression profiling of human angiosarcoma cells with Sprouty2 knockdown together with network data integration using bioinformatics analysis and validated by quantitative PCR revealed important Sprouty2-regulated genes that are involved in angiogenesis, apoptosis and growth signal transduction pathways, including the collagen gene family, Notch signaling pathway and the GTPase IMAP family members.

In summary, these findings demonstrate important growth regulatory role of the FOXO1/Sprouty2 pathway in endothelial cell tumors and highlight the potential roles of novel pathways downstream of Sprouty2 in these lesions.

C5 Therapeutic Targeting of Aldolase A to Inhibit Metastatic Ability via Glycolysis-independent Mechanism in Lung Cancer

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Even metabolic reprogramming regulated transcriptome was appeared to promote cancer metastasis; however, the molecular mechanism remained unclear. Here, we found that glycolysis was accelerated turnover in highly metastatic cancer cells and one of involving enzyme is aldolase A (ALDOA), which forms a positive feedback loop that accelerates glycolysis to produce lactate. Surprisingly, ALDOA forms a glycolysis-independent protein-protein interaction (PPI) with g-actin, which was accompanied by a feedback loop of the phospholipase D1 (PLD1)/β-catenin signaling axis to enhance PLD1 transcription regardless of HIF-1α activity, thus promoting cancer metastatic progression. Moreover, the increased levels of ALDOA and g-actin are commonly detected in malignancies as compared to non-tumor tissues including fibroblast, alveolar cell or bronchial cell. The interaction status also strongly correlated with poorly prognosis and several clinical parameters with patients. Specific peptide had been designed for blocked interaction between ALDOA with g-actin to decrease metastatic ability *in vitro* and prolong survival rate *in vivo* study under low toxicity condition. These findings suggest a new therapeutic strategy of targeting the cancer-associated PPI between ALDOA with g-actin to combat metastatic cancers.

C6 Regorafenib Inhibited MALT1 Expression Through RAF/ERK/ELK1 Pathway in Cholangiocarcinoma

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Background and Aims: Cholangiocarcinoma (CCA) is characterized by aggressive behavior and lack of effective targeted therapies. Regorafenib is a potent oral multi-kinase inhibitor binding to and inhibiting kinases involved in tumor angiogenesis, oncogenesis and tumor microenvironment. However, the similar multi-kinase inhibitors, like sorafenib and sunitinib, have only demonstrated marginal efficacy in clinical trials; therefore, the aim of this study was to investigate the additional mechanism of regorafenib in CCA.

Methods: MTT, Annexin V-FITC apoptosis detection kit, and Western blot analysis were used to evaluate the efficacy of regorafenib in two human CCA cell lines, HuCCT1 and KKU100. Two animal models, Xenograft animal model and TAA induced CCA rat model were used to investigate the drug efficacy in *in vivo* study. Several *in silico* methods, including Library of Integrated Network-based Cellular Signatures (LINCS), PROMO 3.0, and Ingenuity Pathway Analysis (IPA), were used to find the new drug target of regorafenib and related pathway. Finally, we used immunohistochemical staining to evaluate the clinical significance of MALT1 in 100 hepatectomized cholangiocarcinoma patients.

Results: We demonstrated that regorafenib inhibited both HuCCT1 and KKU100 cell growth and induced cell apoptosis. The efficacy of regorafenib was confirmed by two animal models. MALT1, which has an important role in the activation of the transcription factor NF-κB, was identified as a new drug target by LINCS analysis and we found the MALT1 inhibitor, MI-2, also had anti-tumor effect in CCA cells. We further demonstrated regorafenib inhibited NF-κB activity in CCA cells. Regorafenib inhibited MALT1 through its promoter transcription factor ELK-1 as identified by PROMO 3.0 and *IPA* methods. In addition, we also found that overexpression of ELK1 expression in HuCCT1 cells markedly reduced sensitivity to regorafenib, which might be attributed to activate MALT1. Most importantly, MALT1 was also identified as an independent poor prognostic factor for patients with intrahepatic CCA.

Conclusions: Our results indicate that MALT1 plays an important role in cholangiocarcinoma and regorafenib can inhibit MALT1 through the RAF/ERK/ELK1 pathway.

C7 Loss of Function Mutation in *TIMP2* Gene Accelerates Tumorigenesis and Mortality in Mice

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We developed a mouse model with a loss of function mutation in the *TIMP2* gene. This mutation causes diminished expression of TIMP2 and suppression MMP inhibitory activity. We studied tumorigenesis by an orthotopic mouse model of lung cancer using Lewis lung (LL/2) carcinoma cells in these mice.

Mice were given 1x10^6 Lewis lung carcinoma cells transfected with luciferase (LL/2-Luc-M38, Caliper) in 50µL PBS via intratracheal installation. The IVIS analysis shows higher tumor burden in the T2M$^{-/-}$ mice compared to their WT littermates, suggesting that loss of function in the TIMP2 gene amplifies tumor development in these mice ($p<0.05$). In a separate set of experiments, we conducted a Kaplan-Meier analysis to determine the effect of this mutation on mortality following cancer development.

We found that LL/2-induced mortality was significantly higher in the T2M mice compared to the WT controls ($p<0.01$). The H&E analysis of lung tissue sections revealed a significant increase in the number of tumor nodules in the lungs of the T2M mice compared to the WT controls ($p<0.01$). Furthermore, we determined neovascularization by evaluating microvessel density in tumor tissue by calculating the mean number of CD31$^+$ vessels. We found a higher number of CD31$^+$ vessels in the T2M mice compared to the WT mice ($p<0.01$). The vascular endothelial growth factor (VEGF) is primarily a driver of tumor neovascularization, thus we determined the expression of VEGF in healthy and tumor bearing mice. Surprisingly, the basal level of VEGF expression was increased in the lungs of healthy T2M mice compared to WT mice ($p<0.01$), and was even higher in tumors of T2M mice compared WT mice ($p<0.01$). VEGF is the direct downstream target of HIF-2a and the HIF-2a–VEGF signaling pathway plays a major role in cancer progression; as a result, the HIF pathway has become of great interest as a potential strategy for the development of new cancer therapies. High HIF-2a levels in non–small cell lung cancer (NSCLC) correlates with decreased overall survival, while inhibition of HIF targeted genes, such as VEGF or VEGFR2, are associated with improved clinical outcomes. We found TIMP2 mRNA levels to be low in human NSCLC tissues compared to the surrounding non-neoplastic surrounding lung ($p<0.05$). In an effort to investigate the relationship between TIMP2 and HIF-2a expression, we determined the expression levels of HIF-2a in healthy non-tumor bearing T2M mice compared to WT littermates. Using semi-quantitative real-time PCR, we confirmed a strong up regulation of basal HIF-2a mRNA levels in T2M mice lungs compared to WT mice ($p<0.01$). In tumor bearing mice, this increase was even higher in the lungs of the T2M mice compared to WT mice ($p<0.01$). Furthermore, we have investigated the therapeutic potential of recombinant TIMP2 in a triple-negative breast cancer (TNBC) mouse model. The treatment of mice with recombinant TIMP2 significantly inhibited the tumor growth compared to vehicle treated mice.

In conclusion, these findings suggest that TIMP2 regulates hypoxic mediators within the tumor microenvironment and inhibit tumor growth in breast cancer model. Therefore, offering TIMP2 as a novel bio-therapeutic molecule for cancer therapy.

C8 Foxi3 Ablation Retards Bone Derived Prostate Cancer Cell Growth

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Prostate cancer is the abnormal growth of cells in the prostate gland and studies have shown that 100% of men that die from prostate cancer have bone involvement. Of the many cells resident in the bone, myeloid cells secrete various soluble factors that contribute to the high turnover rate of cells and molecular processes of bone development. Of particular interest, Foxi3, a forkhead family transcription factor is critical in bone development and embryogenesis. However, its role in prostate cancer, has not been explored. Therefore, we hypothesized that Foxi3 is a key factor in promoting prostate cancer progression to the bone and its expression is modulated by FGF8. To investigate the clinical role of Foxi3 and study the effect of FGF8 on Foxi3 high expressing cells (PC3 and C42b), we analyzed its expression and function in human prostate cancer tissue and cell lines with or without FGF8 treatment. A significant increase in Foxi3 expression as cancer becomes more aggressive is identified in human specimens ($p<0.01$). Immunohistochemical analysis demonstrated a significant association of Foxi3 expression with tumor grade ($p<0.05$) and pathology ($p<0.01$). Further, C42b cells treated with FGF8 presented an 83-fold increase in Foxi3 expression and a significant increase in migration and proliferation rate ($p<0.001$). Interestingly, Foxi3 inhibition (sh-RNA-Foxi3) in PC3 and C42b cells, significantly decreased cell proliferation and migration ($p<0.01$), even in the presence of FGF8. Together, our findings illustrate a synergistic oncogenic role of Foxi3 and FGF8 in promoting prostate cancer bone metastases.

Keywords: foxi3, bone, prostate cancer, fibroblast growth factor.

C9 Conjugated Bile Acids Promote Esophageal Adenocarcinoma Cell Proliferation via Activation of Sphingosine 1-Phosphate Receptor 2

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Barrett's esophagus (BE) is a premalignant condition that is strongly associated with an increased risk for esophageal adenocarcinoma (EA). The incidence of EA has been continuously and significantly increasing in the United States and other countries during the last four decades. EA is a highly lethal malignancy with an extremely low survival rate. Effective therapy for EA is currently unavailable due to the limited understanding of its pathophysiology. The development of new strategies for the early detection, prevention, and therapeutic interventions for EA is especially urgent, and this is the long-term objective of this research project. It has been reported that BE and EA patients reflux high concentration (>150 µM) of acid into the esophagus as compared to normal individuals (<20 µM). Bile acids are now recognized as important regulatory molecules that activate different cell signaling pathways based on conjugation state and hydrophobicity. Our previous studies reported that conjugated bile acids (CBA), taurocholic acid (TCA) and glycocholic acid (GCA), but not unconjugated bile acids, are able to activate growth promoting and tumorigenic cell signaling pathways by activating the sphingosine-1-phosphate receptor 2 (S1PR2) in cholangiocarcinoma cells. In contrast, unconjugated DCA activates different cell signaling pathways by different mechanisms. However, the role of S1PR2 in bile acid-mediated proliferation and migration of EA cells is not clear and is the focus of this study.

Methods: Studies were conducted in the human EA cell line OE-33. The expression of individual S1PR and sphingosine kinase (SphK) was determined by real-time RT-PCR and confirmed by DNA sequencing. The chemical antagonist of S1PR2, JTE-013, and a gene-specific shRNA were used to inhibit S1PR2 activation and expression. The mRNA and protein levels of target genes were determined by real-time RT-PCR and Western blot analysis, respectively. Cell proliferation was determined using a CCK-8 kit. The Wound-Healing assay was used to examine cell migration.

Results: The S1PR2 and SphK2 are the predominant S1PR and SphK expressed in human OE-33 cells. In addition, S1PR3 and SphK1 are also expressed. Both S1P and CBA (TCA and GCA) induced cell migration and proliferation, which were inhibited by the chemical antagonist of S1PR2, JTE-013. Similar to our findings in human cholangiocarcinoma cells, CBA (TCA/GCA)-induced expression of cyclooxygenase 2 (COX2) was also inhibited by JTE-013. In addition, TCA also significantly induced SphK2 expression, which was blocked by JTE-013.

Discussion/Conclusion: High bile acid level is associated with esophageal carcinogenesis. S1PR1, S1PR2, S1PR3, SphK1 and SphK2 are all expressed in human EA cancer cells. However, S1PR2 and SphK2 expression levels are much higher than those of S1PR1, S1PR3 and SphK1. In this study, by using a chemical antagonist and gene-specific shRNA, we were able to show that S1PR2 plays a critical role in CBA-mediated cell proliferation and migration. The results in this study suggest that CBA promotes EA cell proliferation *via* activation of S1PR2 and SphK2 and targeting S1PR2 may represent a novel therapy for EA.

C10 High-Payload Aptamer-Drug Conjugates for Targeted Pancreatic Cancer Therapy

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Pancreatic cancer is a lethal disease with limited treatment options as specific biomarkers are not currently available for diagnosis, prognosis, and targeted therapy. Therefore, it is urgent to develop an approach to specifically target pancreatic cancer. Aptamers, short RNA or ssDNA

oligonucleotides with high affinity for their targets, have been widely studied in tumor detection and targeted therapy. Moreover, even without knowledge of target molecules, cancer cell-specific aptamers can be developed, successfully.
In this study, ssDNA aptamers specific for pancreatic cancer cells were identified by three rounds of selection with cultured pancreatic tumor cells, followed by 7 rounds of selection with mixtures of pancreatic tumor and non-pancreatic cells. The developed aptamers specifically bound cultured pancreatic tumor cell lines and primary tumor cells with high affinity, but did not bind off-target tumor or normal blood cells. The aptamers were attached to GC repeat sequences, which enhance loading of the chemotherapeutic drug doxorubicin (DOX) to the aptamer via intercalation, thus producing the Aptamer-Drug Conjugates (ADC). Subsequently, ADC tetramers were formulated by employing neutralized avidin and the biotined aptamer containing GC repeat sequences. UV absorption spectroscopy showed that the ADC tetramer was able to incorporate DOX in 20 to 30-fold excess, via non-covalent interactions at GC sites (DOX/tetramer ADC = mol/mol). The formed ADC tetramer was able to specifically target pancreatic cells, and remained stable under normal biological conditions (pH 7.4), indicating its capacity for *in vivo* drug delivery.
Interestingly, our studies showed that the DOX payload could be completely released from the ADC tetramer after DNA degradation within DNase-rich lysosomes. *In vitro* studies showed that ADC specifically targeted pancreatic cells in cell cultures and was rapidly delivered in them in 20 minutes, released DOX in the cytoplasm, and was distributed in the cell nuclei within 2 hours. Treatment with ADC significantly induced growth inhibition of pancreatic cancer cells, but had negligible toxicity to off-target control cells under the same conditions.
These findings demonstrate the potential clinical value of the ADC tetramer in designing (or developing) new personalized medicines for targeted pancreatic cancer therapy.

C11 Aptamer-equipped Natural Killer Cells for Precision Cancer Immunotherapy

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Currently, immunotherapy is one of the most promising strategies for precision cancer therapy, which is safer and more efficient than traditional chemotherapy and radiation treatments. Natural killer (NK) cells are lymphocytes that play critical roles in defense against tumors. They rapidly kill target cells without prior sensitization or major histocompatibility complex restriction. In addition, NK cells have fewer side effects, such as tumor lysis syndrome and graft-versus-host disease responses, which are often seen in T-cell therapy. Although clinical studies have demonstrated the safety and preliminary efficacy of NK cell-adoptive transfer, especially in hematologic malignancies, NK cell therapy lacks cancer cell-type specificity. Here, we report a simple and effective method to engineer NK cells, and increase their specificity to tumor cells.
Aptamers are short, single-stranded oligonucleotides (RNA or ssDNA) that bind to defined targets with high affinity and specificity. They are easily synthesized and modified and display low immunogenicity, features that. render them advantageous and widely applicable in the biomedical field for biomarker discovery, *in vitro* diagnosis, *in vivo* imaging, and targeted therapy. For proof-of-concept, CD30-expressing lymphoma cells were used as a model because CD30 is a cell surface protein that is highly expressed in many malignancies, including Hodgkin lymphoma and anaplastic large cell lymphoma. In addition, our lab has developed aptamers specifically targeting CD30-expressing lymphoma cells.
In order to endow NK cells with specificity to CD30+ lymphoma cells, we engineered NK cells with CD30-specific aptamers on the cell membrane, and thus generated unique aptamer-equipped NK cells. For this purpose, we conjugated the CD30-specific aptamer to the hydrophobic saturated fatty acid chain DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine) through the hydrophilic linker polyethylene glycol (PEG), thus producing the amphiphilic molecule Apt-PEG-DPPE. In aqueous solutions, Apt-PEG-DPPE molecules spontaneously self-assemble into monodispersed, three-dimensional micellar nanostructures with a lipid core, and an ssDNA corona. In the presence of NK cells, however, the micellar assemblies 'open up' and insert onto the cell membranes. Through this physicochemical process, the NK cells are equipped with artificial cell membrane receptors, namely specific ssDNA aptamers. Successful cell engineering was validated with cultured NK92 cell lines and primary NK cells derived from healthy donors. Cultured CD30+ lymphoma cells were used for the cell-targeting study. The aptamer-equipped NK cells showed significantly higher binding affinity to the CD30+ lymphoma cells as compared to parent NK cells. This was evidenced by formation of more NK-target cell clusters when the two cell populations were mixed together. Furthermore, the engineered NK cells exhibited specificity and enhanced cytotoxicity towards CD30+ lymphoma cells.
These findings show that aptamer-equipped NK cells gain binding affinity and specificity for targeted cancer cells, thus opening a new avenue for precision cancer immunotherapy.

C12 Aptamer-induced S Phase Arrest and Priming of Maver-1 Lymphoma Cells for Cytarabine Treatment

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Non-Hodgkin's lymphoma (NHL) is the most common hematological malignancy in adults, with B-cell lymphomas accounting for 85% of all NHLs. B-cell lymphomas are presently treated with chemotherapeutic agents combined with the anti-CD20 monoclonal antibody rituximab. However, rituximab not only depletes lymphoma cells but also normal B cells, which are required for humoral immunity in patients. The ideal therapeutic agent should specifically target lymphoma cells with minimal or no off-target effect on normal B cells. It is well known that B cell lymphoma is a clonal disease and thus lymphoma cells express only one type of surface immunoglobulin light chain. Currently, the lambda clonal light chain, a small polypeptide expressed on lymphoma cells, is the gold standard biomarker for lymphoma diagnosis, and provides a potential target for precision lymphoma therapy.
Aptamers are a class of low molecular weight ligands composed of short, single-stranded oligonucleotides. Similar to antibodies, aptamers are able to recognize and specifically bind to their targets with high affinity. To target lymphoma cells, we developed a DNA aptamer (HL-1) that specifically binds Maver-1 B lymphoma cells. Immunoprecipitation of cellular proteins and mass spectrometry data analysis demonstrated that HL-1 specifically react with Immunoglobulin lambda-like polypeptide of lymphoma cells. Flow cytometry analysis showed that aptamer HL-1 had high binding affinity to Maver-1 lymphoma cells with K_d= 73±9 pmol/L, and showed no reaction to off-target lymphoma cells. To determine the minimal required functional aptamer sequence, different truncated forms of the aptamer were synthesized. Functional assays revealed that the full-length sequence was required for maximal cell binding of aptamer HL-1. Interestingly, exposure of Maver-1 lymphoma cells to synthetic aptamers resulted in growth inhibition by inducing S-phase arrest of 40% cells (vs. 18% baseline in controls), but no detectable growth inhibition was detected in off-target control cells. In light of these findings, we considered the combined use of aptamers with cytarabine, one of the most common chemotherapeutics that selectively affect cells in S-phase. Indeed, *in vitro* studies demonstrated that aptamer-induced S-phase arrest primed Maver-1 lymphoma cells for subsequent chemotherapy: dead cells increased from 12% (aptamer alone) or 14% (cytarabine alone) to 61% for the combined aptamer/cytarabine treatment. These findings lead to a new approach for precision lymphoma therapy, namely aptamer-induced biotherapy alone or combination treatment with chemotherapeutic drugs.

C13 Unique Aptamer-reporting System for Circulating Tumor Cell Detection

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Studies have demonstrated that circulating tumor cells (CTCs) in the bloodstream play a critical role in metastasis, which is the major cause of

mortality in cancer patients. Detection of CTCs is imperative for proper management of cancer patients because the presence of CTCs in blood is directly associated with cancer prognosis. Current CTC assays use antibodies and require multiple steps, which are time-consuming and, more importantly, lead to loss of blood sample and CTC damage. To overcome these technical limitations, we developed a unique approach for rapid CTC detection by taking advantage of aptamer technology and pH-sensitive fluorescence chemistry.

Aptamers are a class of low molecular weight ligands composed of short, single-stranded oligonucleotides. Similar to antibodies, aptamers are able to recognize and specifically bind to their targets with high affinity. Aptamer probes labeled with fluorescent dyes have been widely studied for specific detection of cancer cells. However, these aptamer probes constantly emit a fluorescent signal regardless of their cell- bound or free state, leading to a high level of background noise. Due to this, repeated washes are required to remove excess free fluorochrome-conjugated aptamer probes from the assays, a process which may lead to loss and damage of CTCs. To overcome these technical obstacles, our lab developed a cancer cell-activatable aptamer-reporting system, which was formulated by conjugating the aptamer with paired fluorochrome-quencher molecules. Upon intracellular degradation of the aptamer, the fluorochrome is separated from the paired quencher molecule and emits a fluorescent signal, thus highlighting CTCs for detection in whole blood.For further development, we designed a 2nd generation aptamer-reporting system by conjugating the EpCAM-specific aptamer to a pH-sensitive fluorochrome, which is optically silent at normal pH and fluorogenic when pH is ≤ 5.0. The EpCAM-specific aptamer that we developed is cancer cell-specific and pH-activated.

Cell assays demonstrated that when the EpCAM-specific aptamer was added to cell cultures, the aptamer-reporter selectively bound target cancer cells and triggered subsequent internalization into cell lysosomes. The acidic microenvironment of the cell lysosomes (pH 4.8) rapidly activated the pH-sensitive fluorochrome of aptamer-reporter, resulting in fluorogenesis exclusively within the EpCAM-expressing cancer cells, but not in off-target control cells.

Notably, no background signal was emitted during the cell assays because the free aptamer-reporter molecules were optically silent. The 2nd generation aptamer-reporter was able to highlight rare carcinoma cells in whole blood while avoiding emission of off-target signal. Our results provide a new approach to detect CTCs in whole blood with high sensitivity and specificity, in a high-throughput fashion.

C14 Innovative Aptamer-guided Highlighter for Real-Time Tumor Detection and Guided Resection

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The primary treatment of solid cancers entails surgical resection of tumor mass followed by chemotherapy. Complete tumor resection is critical to increase patient overall survival and decrease recurrence. However, complete resection is often challenging, as tumors can be morphologically indistinguishable from normal tissues/organs. Current frequently used imaging technologies for tumor detection include CT, MRI, and PET/CT, but these methods lack tumor specificity, require special equipment/facilities, and produce harmful radiation for patients and clinicians. Visible dyes, such as blue dye-guided sentinel lymph node identification, are used to guide surgical procedures, but all of them lack tumor specificity as well. To overcome the technical obstacles of conventional imaging methods in tumor detection and address clinical needs, we developed and tested an innovative cancer-specific and tumor-cell-activated highlighter by combining the advantages of aptamer technology and fluorescence-based techniques, which provide high signal-to-background ratios and thus significantly improve the sensitivity of tumor detection.

Aptamers are small ligands composed of short oligonucleotides (RNA or ssDNA) that are able to specifically bind to their targets with high affinity. The tumor highlighter that we developed was formulated by conjugating a biomarker-specific aptamer to a pH-sensitive fluorescent reporter, which is optically silent under physiological conditions (pH 7.4). After systemic administration, the aptamer of the tumor highlighter selectively binds cancer cells and induces internalization into cell lysosomes (pH 4.8) where the pH-sensitive fluorochrome is rapidly activated and illuminated. The selective identification of the highlighted tumors during surgery enables complete tumor resection under real-time imaging guidance.

For proof-of-concept, we formulated the tumor highlighter by conjugating the CD30-specific aptamer to the pH-sensitive CypH-1 fluorochrome, which fluoresces when the environment pH is ≤ 5.0. In the feasibility studies, CD30-expressing Karpas 299 lymphoma cells with luciferase reporter were used. Addition of the CD30-specific aptamer tumor highlighter to cultures specifically highlighted Karpas 299 cells, but not CD30-negative control cells. For *in vivo* studies, the xenograft mouse model bearing intraperitoneal lymphomas was established. The development of xenograft tumors was monitored by whole body bioluminescence imaging. After intravenously injected with the tumor highlighter, the mice were euthanized and an abdominal incision was performed for tumor detection.

Our study revealed the possibility, via the specific signal emitted from the tumor highlighter, to detect tumors less than 2 mm in diameter, below the detection limit of current imaging methods and hardly visible by the naked eye. Importantly, the highlighted tiny tumors were able to be precisely resected. The complete tumor resection was confirmed by post-operation imaging and histology examination. In summary, we formulated a highly specific aptamer-guided tumor highlighter that is 4 times more sensitive than current imaging methods for *in vivo* tumor detection: the tumor size detection limit for our highlighter is less than 2 mm vs. 8 mm or higher for current methods. Importantly, the tumor highlighter for imaging that we developed will also allow surgeons to conduct real-time guided tumor resection.

CARDIOMYOPATHY

CM1 Inhibiting the Myocyte-Specific Ubiquitin Ligase MuRF1 (Muscle Ring Finger-1) Attenuates Acute Doxorubicin-Induced Cardiomyopathy *In Vivo*

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Doxorubicin (DOX) is used widely for its potent anti-cancer activities and is known to induce a delayed chronic cardiotoxicity associated with cumulative doses. In contrast, acute DOX cardiotoxicity can occur within 2-3 days of administration in ~11% of patients and has been associated with loss of muscle mass. The mechanisms underlying these changes are unknown. We recently identified that the muscle-specific ubiquitin ligase MuRF1 mediates cardiac atrophy *in vivo* using MuRF1-/- mice through its role in specifically ubiquitinating critical sarcomere proteins (e.g. myosin heavy chain, cTnI, cMBP-C), which are targeted for degradation by the proteasome. We hypothesized that MuRF1 contributes to both the acute DOX-induced atrophy and cardiac dysfunction seen *in vivo*. To test this hypothesis, we serially analyzed eight week MuRF1-/- mice for cardiac function by high-resolution conscious echocardiography at baseline and seven days after treatment with 20 mg/kg DOX i.p. No functional or morphological differences were seen between MuRF1+/+ and sibling MuRF1-/- hearts prior to DOX treatment (Ejection Fraction%=83.9$\pm$2.4 vs. 84.3$\pm$1.5, respectively; LV Mass/Body weight=5.0$\pm$0.7 vs. 4.4$\pm$0.3, respectively). After seven days, MuRF1+/+ exhibited both a loss in systolic function (EF%=68.5$\pm$1.7) and cardiac atrophy (LV Mass/Body weight=3.8$\pm$0.2). MuRF1+/+ hearts exhibited an increase in MuRF1 expression commonly seen in both skeletal and cardiac muscle atrophy. In contrast, MuRF1-/- hearts exhibited an attenuated loss of systolic function (EF%=78.1$\pm$4.1) and LV Mass (LV Mass/BW 4.5$\pm$0.10) at seven days post-DOX treatment. We recently identified that MuRF1-/- mice exhibit an athletic phenotype at baseline, running farther and faster due to enhanced cardiac IGF-1 signaling. Specifically, MuRF1-/- mice lack MuRF1's direct inhibition of c-Jun through proteasome-dependent degradation, which results in increased c-Jun and a transcription upregulation of the IGF-1 signaling pathway. Taken together, we hypothesize that blocking MuRF1 activity offers protection from acute

doxorubicin-induced dysfunction and atrophy via its regulation of IGF-1 signaling in addition to its role in degrading the sarcomere. Since sarcomere degradation is the key mechanism that mediates the reduction in cardiomyocyte size in atrophy, blocking MuRF1 clinically prior to DOX treatment may offer a unique target to attenuate DOX-associated morbidity and mortality in the unpredictable subset of patients adversely affected by acute toxicity.

CM2 Muscle Ring Finger-1 Knock-out (MuRF1-/-) Mice are Resistant to LPS-induced Cardiac Dysfunction *In Vivo* Due Partly to Significantly Increased Cardiac PPARalpha Activity Competitively Inhibiting NF-κB

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Sepsis is associated with a high morbidity and mortality (up to 40%) in hospitalized patients. Cardiac dysfunction plays a critical role in the outcome of severe sepsis, and septic shock specific therapies targeting sepsis-associated cardiac dysfunction are lacking. The association between myocardial dysfunction and sepsis was first described in 1984, with subsequent studies identifying the role of Toll-like receptors, cytokines, and NF-κB signaling in the severe heart failure that results. We recently identified that the muscle-specific ubiquitin ligase MuRF1 (Muscle Ring Ringer-1) inhibits the peroxisome proliferator-activated receptor alpha (PPAR-α) in cardiomyocytes by mediating its nuclear export in a ubiquitin-dependent manner. We found that MuRF1 interacts with PPAR-α to mediate a multi-monoubiquitination using three lysines surrounding a newly identified nuclear export sequence. Interestingly, MuRF1 cardiomyocyte transgenic mice have a significant reduction in PPAR--α, whereas MuRF1-/- mice have a five-fold increase in cardiac PPAR-α activity. Since studies in cancer cells have shown that PPAR-α competitively inhibits NF-κB and downstream inflammation, we hypothesized that MuRF1-/- mice would have particularly potent cardiomyocyte anti-inflammatory responses by its competitive 5-fold increase in PPAR-α activity. To test this, we performed echocardiography on eight-week old male and female MuRF1-/- (N=8) and sibling wildtype mice (N=9) at baseline and after at 4, 24, 48, and 72 hours post-lipopolysaccharide injection (2 mg/kg of LPS intraperitoneally). As a potent Tlr-4-like receptor agonist, the gram negative bacterial cell wall (LPS) interacts with Tlr-4-like receptors on cardiomyocytes and inflammatory cells, releasing cardio-depressant cytokines (e.g. TNF-α, IL-1, IL-6) that act upon receptors to activate NF-κB. No mice from either group died during the 72-hour experiment. At baseline, MuRF1-/- hearts did not differ in function or size at baseline compared to wildtype sibling controls (Ejection Fraction (EF)%=85.7$\pm$1.0 vs. 87.7$\pm$0.5, respectively; LV Mass/Body weight=5.51$\pm$0.4 vs. 5.61$\pm$0.32, respectively). Four hours after LPS, wildtype mice exhibited significantly more systolic dysfunction compared to wildtype mice (EF%=67.6$\pm$2.2 vs. 78.5$\pm$0.9, respectively). Wildtype hearts exhibited anterior and posterior wall thinning at 4 hours (both 0.83$\pm$0.04 vs. 0.91$\pm$0.03 mm at baseline), whereas MuRF1-/- hearts wall thickness did not change from baseline. The decrease in heart size, however, was comparable in the MuRF1-/- and wildtype mice, both losing LV Mass/Body weight of ~0.5 mg/g which may be due to water loss and/or reduced food/water intake due to LPS-induced behavioral changes in appetite. The LPS-induced cardiac dysfunction in MuRF1-/- hearts was completely resolved by 72 hours (EF%=80.14$\pm$1.53), whereas wildtype hearts still exhibited residual dysfunction (EF%=69.2$\pm$2.2).
Together, these studies demonstrate MuRF1's regulation of PPAR-α may affect the heart's ability to respond to NF-κB. Therapeutic inhibition of the MuRF1-PPAR-α interaction provides a muscle-specific NF-κB inhibition that may prove beneficial in NF-κB-mediated cardiac dysfunction seen in sepsis, heart failure, and myocardial infarction.

GENETIC DISEASES

G1 Genetic Treatment of Sickle Cell Disease by Using Biocompatible CRISPR/Cas9 Method and Patient Blood-derived Hematopoietic Progenitor Cells

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Sickle cell disease (SCD) is an inherited form of anemia, which is caused by the point mutation (nt. 69A>T in exon1) in the hemoglobin-beta gene. In red blood cells of SCD patients, the abnormal hemoglobin-S (Hb S) molecules stick together when they release oxygen to the tissues. These clumps cause red blood cells to become stiff and sticky, and assume the shape of sickles or crescent moons. These irregularly-shaped red blood cells can stick in small blood vessels; this can slow down or block blood flow and oxygen to parts of the body. Allogeneic bone marrow transplant offers the only potential cure for sickle cell anemia. However, the marrow transplant procedure has serious risks associated with it, including death. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs), which consist of identical repeated DNA sequences (repeats) interspaced by highly variable sequences, and CRISPR-associated (Cas) genes were discovered in the 1980s. To date, the CRISPR/Cas9 system has been widely adopted as a genome -editing and -targeting tool. This mechanism has already been successfully used to target important genes in many cell lines and organisms. Several groups have now taken advantage of this method to introduce single point mutations (deletions or insertions) in a particular target gene, via a single gRNA. It is also possible to induce large deletions or genomic rearrangement, such as inversions or translocations, by using a pair of gRNA-directed Cas9 nucleases. Use of DNA-based Cas9, RNA-guided vector expression, or Homology Directed Repair (HDR) template vector, however, carries the possibility of undesirable genetic alterations due to random plasmid DNA or lentiviral vector integrations. Another concern is that the final edited product comprises a pool with corrected and uncorrected cells. Despite these obstacles, which have impeded application of CRISPR in clinical therapy, CRISPR studies to correct the SCD mutation have been reported.
In this study, we optimized the method of integration-free CRISPR/Cas9 system to correct the SCD gene in cultured cells. Electroporation was conducted to introduce CRISPR/Cas9 system into cells, and a Taqman RT-PCR assay was established to monitor genetic correction of treated cells. After validation studies with cultured cells, we corrected the genetic mutation of Hb S in hematopoietic stem/progenitor cells (HSPCs), derived from the peripheral blood of a patient who had SCD. Successful genetic correction was demonstrated by Taqman RT-PCR assay, and further confirmed by Sanger sequencing of targeted Hb S gene.
Notably, expression of normal adult hemoglobin HbA was also confirmed by high-pressure liquid chromatography (HPLC) in individual erythroid colonies of genetically corrected HSPCs from SCD patients. Finally, individual genetically corrected erythroid colonies were expanded and re-establishment of red blood cell functions was confirmed.
In summary, our study demonstrates a new approach to treat SCD through genetic correction by using an entirely biocompatible CRISPR/Cas9 method and HSPCs from patient blood.

G2 Vascular Dysfunction in the MDX Mouse

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Muscular dystrophy is a family of diseases caused by mutations in genes encoding proteins that form the dystrophin-glycoprotein complex (DGC) and lead to skeletal and cardiac muscle dysfunction. The skeletal muscle and cardiac muscle pathologies associated with these mutations are well documented, but more recently it has become appreciated that components of the DGC are expressed in vascular smooth muscle and endothelial cells. The physiological role of the DGC in these cells is not well understood. Several groups have postulated that disruption of the DGC in vascular cells contributes to the abnormal vascular responses and blood flow patterns seen in human patients and animal models of muscular dystrophy. Some studies even suggest that vascular

dysfunction contributes to the cardiac and skeletal muscle dysfunction seen in some forms of muscular dystrophy, however, few of these studies directly tested vascular function. In light of the paucity of data regarding the role of the DGC in vascular physiology and pathophysiology, we sought to determine the extent and location of vascular dysfunction in muscular dystrophy by studying vascular segments *in vitro.* Vascular segments were harvested from the MDX mouse, a murine model of Duchenne Muscular Dystrophy, and studied as isometric ring preparations or as pressurized resistance artery segments. We will share the results of our experiments and discuss recent findings from other groups examining the potential roles of the DGC in vascular function.

INFECTIOUS DISEASES

ID1 Altered Mitochondrial and Calcium Metabolism and Apoptosis Contribute to Zika Virus-mediated Cell Injury.

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Zika Virus (ZIKV) is an important human pathogen and is linked to fetal developmental abnormalities such as microcephaly, eye defects, and impaired growth. ZIKV is a member of the Flaviviridae family, and has been shown to be transmitted through vectors, such as mosquitoes, sexual contact, and a vertical route from the infected mother to the fetus. To date, there are no reliable treatments or vaccines options available to protect those infected by ZIKV. Pathobiology of ZIKV-induced cell injury is poorly understood. Here, we provide a molecular basis of cellular changes affected by a contemporary ZIKV strain using an *in vitro* infectious cell culture system. ZIKV infection causes overt cytopathic effects, including changes in cellular morphology and cell lysis, which coincides with the activation of immune and inflammatory genes.

ZIKV infection led to apoptotic cell death, and pharmacological inhibition of caspase-dependent apoptosis did not affect ZIKV replication and plaque formation. This suggested that the programmed cell death is a late stage event triggered by the host in response to the infection. Infected cells showed higher mitochondrial membrane potential and increased intra-cellular calcium level suggesting significant alterations in intracellular metabolism.

In conclusion, dysregulation of mitochondrial function, intracellular calcium over load and late stage programed cell death contribute to cell injury caused by Zika viral infection.

ID2 Dysregulation of Long Non-coding RNA (lncRNA) Genes and Predicted lncRNA-Protein Interactions during Zika Virus Infection

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Zika Virus (ZIKV) is a causative agent for poor pregnancy outcome and fetal developmental abnormalities, including microcephaly and eye defects. As a result, ZIKV is now a confirmed teratogen. Understanding host-pathogen interactions, specifically cellular perturbations caused by ZIKV, can provide novel therapeutic targets. In order to complete viral replication, viral pathogens control the host cellular machineries and regulate various factors, including long non-coding RNA (lncRNA) genes, at transcriptional levels.

The role of lncRNA genes in the pathogenesis of ZIKV-mediated microcephaly and eye defects is currently unknown. To gain additional insights, we focused on profiling the differentially expressed lncRNA genes during ZIKV infection in mammalian cells.

For this study, we employed a contemporary clinical Zika viral isolate, PRVABC59, of Asian genotype. We utilized an unbiased RNA sequencing approach to profile the lncRNA transcriptome in ZIKV infected Vero cells. We identified a total of 121 lncRNA genes that are differentially regulated at 48 hours post-infection. The majority of these genes are independently validated by reverse-transcription qPCR.

A notable observation was that the lncRNAs, MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) and NEAT1 (Nuclear Paraspeckle Assembly Transcript 1), are down-regulated upon ZIKV infection. MALAT1 and NEAT1 are known as nuclear localized RNAs that regulate gene expression and cell proliferation. Protein-lncRNA interaction maps revealed that MALAT1 and NEAT1 share common interacting partners and form a larger network comprising of 71 cellular factors. ZIKV-mediated dysregulation of these two regulatory lncRNAs can alter the expression of respective target genes and associated biological functions, an important one being cell division.

In conclusion, this investigation is the first to provide insight into the biological connection of lncRNAs and ZIKV which can be further explored for developing antiviral therapy and understanding fetal developmental processes.

ID3 Intergenic Variable-Number Tandem-Repeat Polymorphism Upstream of *rocA* Alters Toxin Production and Enhances Virulence in *Streptococcus pyogenes*

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Variable-number tandem-repeat (VNTR) polymorphisms are ubiquitous in bacteria. However, only a small fraction of them has been functionally studied. Here, we report an intergenic VNTR polymorphism that confers an altered level of toxin production and increased virulence in *Streptococcus pyogenes*. The nature of the polymorphism is a one-unit deletion in a three-tandemrepeat locus upstream of the *rocA* gene encoding a sensor kinase. *S. pyogenes* strains with this type of polymorphism cause human infection and produce significantly larger amounts of the secreted cytotoxins *S. pyogenes* NADase (SPN) and streptolysin O (SLO).

Using isogenic mutant strains, we demonstrate that deleting one or more units of the tandem repeats abolished RocA production, reduced CovR phosphorylation, derepressed multiple CovR-regulated virulence factors (such as SPN and SLO), and increased virulence in a mouse model of necrotizing fasciitis. The phenotypic effect of the VNTR polymorphism was nearly the same as that of inactivating the *rocA* gene.

In summary, we identified and characterized an intergenic VNTR polymorphism in *S. pyogenes* that affects toxin production and virulence. These new findings enhance understanding of rocA biology and the function of VNTR polymorphisms in *S. pyogenes*.

ID4 Genomic Analysis of *Staphylococcus aureus* USA300 Clinical Isolates Identifies Naturally Occurring Polymorphisms in the *sarZ* Regulator Gene that Influence Virulence

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Staphylococcus aureus is a ubiquitous pathogen associated with high morbidity and mortality rates. Methicillin-resistant *S. aureus* (MRSA) was once considered primarily a nosocomial pathogen, but is now prominent in healthcare and community settings. The emergence and spread of the community-associated MRSA clone USA300 emphasizes the urgent need for improved understanding of pathogenesis and development of vaccines and novel antimicrobials.

To better understand the success of USA300 as a human pathogen, we sequenced the genome of 981 clinical USA300 isolates. Unexpectedly, we discovered a high prevalence of naturally occurring single nucleotide polymorphisms in the virulence regulator *sarZ*, which is known to control expression of secreted proteases (e.g., SspA) and α-hemolysin. Based on a milk plate assay, 90% of the amino acid replacements in the *sarZ* variants abolished detectable USA300 secreted protease production, and the *sarZ* variants had varied capacity to form biofilms *in vitro*. Compared to the USA300 wild-type strain, an isogenic *sarZ* deletion strain had significantly reduced virulence in mouse models of soft tissue and invasive intramuscular infection.

We hypothesize that naturally occurring mutations in *sarZ* are caused by selective pressure to attenuate the high virulence capacity of USA300. Adaptations towards decreased virulence that maintain the ability to cause invasive infections are a developing area of *S. aureus* research. Additional studies are warranted to determine the benefit of SarZ amino acid replacements in the success of USA300 as human pathogen or commensal organism.

ID5 Rift Valley Fever Virus Growth Curve Kinetics in Cattle and Sheep Peripheral Blood Monocyte Derived Macrophages

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Rift Valley Fever Virus (RVFV), genus *Phlebovirus*, family *Bunyaviridae*, is a single-stranded, negative sense RNA virus endemic to sub-Saharan Africa and the Arabian Peninsula. It causes disease in ruminant species, rapidly reaching high titers and causing nearly 100% abortion and mortality in young animals. The virus also infects humans, with symptoms ranging in severity from self-limiting mild fever to encephalitis, hemorrhagic fever, sometimes resulting in death. Because of the widespread competence of mosquito vector species, there is concern that the virus may become a threat in other regions of the world. When an RVFV-infected mosquito takes a blood meal, macrophages will present viral antigen to lymphocytes, initiating an adaptive immune response. These macrophages are thought to be targets of infection themselves, and may be exploited by the virus to aid in its dispersal. The purpose of this work is to develop a primary macrophage *in vitro* model for screening virus modulatory compounds such as mosquito saliva. Here we present growth curves for Rift Valley Fever Virus strain MP 12 in bovine and ovine monocyte-derived macrophages. Whole blood is collected from healthy animals housed at Kansas State University. Monocytes are cultivated for a period of 7 days at 37^{0}C in 5% CO_2 in T25 flasks in RMPI 1640 media with glutamine, 5% FBS and 1X PSF for differentiation into macrophages. Cells are infected at a multiplicity of infection (MOI) of 0.1. Twenty percent of the supernatant is collected and exchanged with fresh media every 8 hours for 96 hours. Viral RNA is isolated from supernatant using Trizol and magnetic bead extraction methodology under automation (MagMax Express, Thermofisher). Quantitative reverse transcription real-time polymerase chain reaction (RT-qPCR) detecting RVFV L and M segments is used to determine viral copy number. Virus titers are determined utilizing a standard plaque assay on Vero cells.
Preliminary data demonstrate bimodal virus replication (based on viral copy number) during the 96 hour period. The initial replication peak occurred at 8-16 hours in cattle and 16-32 hours in sheep. A second period of virus replication occurred at 64-72 hours in both species. In 3 out of 4 sheep, the viral copy number maximums in both replication cycles are 1-2 logs higher than those of cattle ($3 \times 10^4 – 2.5 \times 10^6$ for sheep, and $1.8 \times 10^4 – 3.5 \times 10^4$ for cattle). In sheep, the second peak nearly reaches the magnitude of the first peak, but in cattle, the magnitude of the second peak is markedly decreased (50-75%).
To better understand RVFV replication in macrophages, additional experiments using alternative MOIs and supernatant collection methods will be conducted. In addition, immunofluorescence and plaque assays are being performed. Further experimentation is planned to examine the effects of mosquito saliva on viral replication using this model.

ID6 Pathology Review of Two New Rift Valley Fever Virus Ruminant Models

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Rift Valley fever virus (RVFV), is a mosquito-borne, zoonotic pathogen within genus *Phlebovirus*, family *Bunyaviridae* that typically causes outbreaks in sub-Saharan Africa and recently spread to the Arabian Peninsula. In ruminants, RVFV infections cause mass abortion and high mortality rates in neonates. In humans, Rift Valley fever (RVF) often presents as an acute febrile illness but may progress to severe disease, including retinal vasculitis, encephalitis, hepatitis, hemorrhagic fever and death. There is no antiviral therapy. Both Europe and North America have experimentally competent RVFV vectors. However, they have no fully licensed vaccines for use in ruminants or humans. In endemic countries the standard veterinary control approach is vaccination with attenuated vaccines. However, these vaccines have the potential for teratogenicity as well as reversion to virulence or reassortment with RVFV field strains. They are not differentiating infected from vaccinated (DIVA) compliant and therefore unlikely to be approved for use during an epizootic in the US or Europe. The existence of naïve economically important ruminant populations in Europe and North America, the human disease risk and the potential to weaponize RVFV warrant a One Health approach to the development of vaccine and other control strategies.
We developed sheep and cattle challenge models for RVF. These are unique in that they are subcutaneous inoculation models that reliably produce clinical disease in juevniles. We compared two genetically-distinct RVFV strains, Saudi Arabia 2001 and Kenya 2006 (Ken06). We demonstrated differences in viremia and clinico-pathological changes. Development of these challenge models and our more recent use of the Ken06 sheep model for vaccine efficacy testing afforded examination of RVFV pathology in multiple organs.
In the liver, we saw a range of lesions from a mild lymphohistiocytic hepatitis with rare single hepatocyte apoptosis to severe, multifocal central to midzonal necrosis. At 3 to 5 days post-infection (dpi) liver lesions were consistently positive for RVFV antigen by immunohistochemistry. Occasionally, in Ken06 infections we saw glomerular filtration of RVFV antigen at 3-5 dpi and renal tubular necrosis and degeneration in which epithelial cells and debris were viral antigen positive at 10 dpi. Splenic lesions ranged from mild, scattered lymphoid follicular depletion to marked lymphocytolysis affecting both the white and red pulp that was RVFV antigen positive. Sporadically, we saw RVFV antigen positive lesions in other organs. Occasionally, individual animals exhibited signs and lesions consistent with disseminated intravascular coagulation. We also found RVFV antigen positive macrophages in lymph nodes and less commonly, the lungs. Although our sample sizes were small, 10-12 animals per study, we have appreciated virus, infection time-course and species differences in pathology.
Continuing lines of enquiry using these study materials along with comparisons to natural disease case material provided through a South African collaboration will enable us to examine open questions regarding RVF viral pathogenesis and host response. Parallel work wherein we established the LD50 for multiple RVFV strains in Balb/c mice including a preliminary pathology review, will enable us to study viral pathogenesis and immunopathology questions in an animal model for which there are abundant reagents.

ID7 Transcriptome Remodeling Contributes to Epidemic Disease Caused by the Human Pathogen *Streptococcus pyogenes*

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For over a century, a fundamental objective in infection biology research has been to understand the molecular processes contributing to the origin and perpetuation of epidemics. Divergent hypotheses have emerged concerning the extent to which environmental events or pathogen evolution dominates in these processes. Remarkably few studies bear on this important issue. Based on population pathogenomic analysis of 1,200 *Streptococcus pyogenes* type *emm*89 infection isolates, we report that a series of horizontal gene transfer events produced a new pathogenic genotype with increased ability to cause infection, leading to an epidemic wave of disease on at least two continents. In the aggregate, these and other genetic changes substantially remodeled the transcriptomes of the evolved progeny, causing extensive differential expression of virulence genes and altered pathogen-host interaction,

including enhanced immune evasion. Our findings delineate the precise molecular genetic changes that occurred and enhance our understanding of the evolutionary processes that contribute to the emergence and persistence of epidemically successful pathogen clones. The data have significant implications for understanding bacterial epidemics and for translational research efforts to blunt their detrimental effects.

IMMUNOLOGY

IM1 A Novel Injury Site-Specific Complement Inhibitor Reduces Neuronal Loss and Prevents Chronic Inflammation after Murine Stroke

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Ischemia leads to the cellular expression of damage-associated molecular patterns (DAMPS), and their recognition by natural IgM antibodies leads to the activation of complement and propagation of inflammation. We previously isolated two natural IgM monoclonal antibodies (mAbs) from un-manipulated mice: B4 that recognizes modified annexin-IV, and C2 that recognizes a subset of phospholipids. Both B4 and C2-mAbs specifically recognized ischemic cells and reconstituted cerebral ischemia reperfusion injury in otherwise protected antibody-deficient Rag1-/- mice. Based on this, we developed a strategy for site-targeted complement inhibition by fusing a single chain antibody (scFv) derived from the B4-mAb to the complement inhibitor Crry. We show that the fusion construct, B4scFv-Crry, inhibits complement activation *in vitro* and targets specifically the ischemic brain *in vivo* following 60 mins transient middle cerebral artery occlusion (MCAO).

A single dose of B4scFv-Crry administered 6 hrs after ischemia inhibited pathogenic IgM and complement deposition in the ischemic brain, resulting in significantly reduced infarct volumes and neurological deficit scores in male, female and aged mice, as measured 24 hrs after ischemia. B4scFv alone provided similar levels of improvement at 24 hrs after ischemia, indicating the targeting vehicle may play a protective role by inhibiting the binding of self-reactive pathogenic Abs. However, only B4scFv-Crry provided protection into the chronic phase (15 days), yielding a significant reduction in cell death and tissue scarring, and significant improvement in gross motor deficits, forelimb asymmetry and cognitive performance on Barnes maze and passive avoidance tasks. Using high-resolution immunofluorescent staining, we also demonstrated complement deposition on live penumbral neurons and their subsequent uptake by reactive microglia, a process leading to rapid neuronal loss after stroke. Inhibition of complement by B4scFv-Crry significantly inhibited the phagocytosis of live penumbral neurons and resulted in higher neuronal density in the penumbra. Interestingly, B4scFv-Crry treatment did not interfere with apoptotic cell clearance. Following acute MCAO, we observed a sustained neuro-inflammatory response manifesting as continuous IgM and complement deposition and robust M1 (pro-inflammatory)-type activation of microglia lasting beyond 15 days of reperfusion. Acute administration of B4scFv-Crry after stroke interrupted the inflammatory neurodegenerative cycle by significantly inhibiting complement and IgM deposition, and shifting microglial polarity to resting and M2-polarized phenotypes in the chronic phase. This effect removed the brake on regenerative mechanism, and explains the pronounced increase in neurogenesis and neuronal migration in B4scFv-Crry treated animals. Finally, we demonstrated that both B4-scFv and B4scFv-Crry bound specifically within the ischemic core and penumbra of brain samples obtained from patients who died after acute stroke. No binding was observed in sections prepared from norma brain tissue from the same patient, or from age-matched controls.

These data indicate that a similar DAMP recognition system occurs in the brains of mouse and man, and that B4scFv-targeted complement inhibition has translational potential for inhibiting inflammatory neurodegenerative cascades after stroke.

IM2 Depletion of Major Pathogenic Cells in Asthma by Targeting CRTh2

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Eosinophilic inflammation and Th2-cytokines contribute to asthma pathogenesis. Agents targeting either eosinophils or single Th2-cytokines have shown efficacy in distinct subsets of asthma patients; targeting multiple inflammatory stimulators simultaneously may broaden disease modifying effects and efficacy. Here we present a therapeutic strategy to concomitantly deplete Th2 T-cells, eosinophils, basophils, and type-2 innate lymphoid cells (ILC2s) using a monoclonal antibody with enhanced effector function (mAb 19A2) that targets a molecule expressed on all four cell-types, chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2). Transgenic mice recapitulating the human expression pattern of hCRTH2 on innate immune cells but not Th2-cells show specific antibody-mediated elimination of hCRTh2$^+$ basophils, eosinophils and ILC2s from lung and lymphoid organs in models of asthma and *Nippostrongylus brasiliensis* infection; decreases of multiple Th2-cytokines and chemokines accompany mAb 19A2-dependent hCRTH2$^+$ innate immune cell depletion. mAb 19A2 shows *in vivo* activity on human Th2-cells in a human Th2-PBMC-SCID mouse model. Humanized mAb 19A2 (h19A2) potently induces antibody-dependent cell-mediated cytotoxicity (ADCC) of primary human eosinophils and basophils *in vitro*, emulating the depletion capacity of the murine parent antibody *in vivo*.

Depletion of hCRTh2$^+$ basophils, eosinophils, ILC2 and Th2-cells with h19A2 is a novel and potentially efficacious treatment for asthma.

IM3 Preclinical Safety Profile of a Depleting Antibody Against CRTh2 for Asthma: Well Tolerated Despite Unexpected CRTh2 Expression onVascular Pericytes in the Central Nervous System and Gastric Mucosa

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CRTh2 is expressed on immune cells that drive asthma pathophysiology. Current treatment options for severe asthma are inadequate and therapeutic antibody-mediated depletion of CRTh2-expressing cells represents a promising new therapeutic strategy. Here we report for the first time that CRTh2 is not only expressed on immune cells, but also on microvasculature in the central nervous system (CNS) and gastric mucosa in humans. Microvascular expression of CRTh2 raises a safety concern because a therapeutic anti-CRTh2 antibody with enhanced depletion capacity could lead to vascular damage. To evaluate this safety risk, we characterized microvascular expression in human and in transgenic mice expressing human CRTh2 protein (hCRTh2.BAC.Tg) and found that CRTh2 is not localized to microvascular endothelium that is directly exposed to circulating therapeutic antibody, but rather to pericytes that in the CNS are shielded from direct circulatory exposure by the blood-brain barrier. Immunohistochemical visualization of an intravenously administered anti-CRTh2 antibody in transgenic mice revealed localization to microvascular pericytes in the gastric mucosa but not in the CNS, suggesting the blood-brain barrier effectively limits pericyte exposure to circulating therapeutic antibody in the CNS. Repeated dosing with a depleting anti-CRTh2 antibody in hCRTh2.BAC. Tg mice revealed linear pharmacokinetics and no drug-related adverse findings in any tissues, including the CNS and gastric mucosa, despite complete depletion of CRTh2 expressing circulating eosinophils and basophils. Collectively, these studies demonstrate that the likelihood of drug-related CNS or gastrointestinal toxicity in humans treated with a therapeutic depleting anti-CRTh2 antibody is low despite pericyte expression of CRTh2 in these tissues.

IM4 Characterization of Cutaneous Expression of Interleukin-2 in Lupus-Model Mice

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Background and Significance: Systemic lupus erythematosus (SLE) is an autoimmune disease that damages multiple systems in the body, notably the skin. Skin lesions in SLE patients include impaired wound healing, which is characterized by both delayed healing and excessive scarring at wound sites, represent a source of morbidity for these patients and warrant exploration. The blood of patients with SLE is known to be deficient in Interleukin-2 (IL-2), which impacts immune cell development and inflammation. IL-2 is implicated in wound healing, specifically by increasing the strength of healed skin. However, the impact of IL-2 signaling in the cutaneous manifestations of SLE is not known. Because IL-2 impacts wound healing and because SLE includes significant skin manifestations, the potential contribution of IL-2 to impaired healing in SLE warrants further study.

Hypothesis: Wound healing in SLE is abnormal due to aberrant IL-2 signaling.

Methods: B6.MRL-Fas<lpr>/J (LPR) mice served as the model of SLE with the C57BL/6J background strain as the control. Following a biopsy punch wound, mice were allowed to heal for 10 days. Wounds were analyzed using Masson's trichrome stain and Immunohistochemistry (IHC) for IL-2. This data was correlated with human skin microarray data from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) Database. L929 fibroblast cells grown with and without a gelatin and fibronectin matrix and treated with recombinant IL-2 served as a model to further explore IL-2-mediated alterations in wound healing. Statistical analysis includes ANOVA and t-tests where appropriate.

Results: WT and LPR mice heal wounds at the same rate. Trichrome stains reveal that the uninjured skin LPR mice have disorganized skin fiber formation compared to controls. LPR wound sites appear to exhibit decreased fiber formation, which may indicate an impaired closure of the wound or a delay in collagen deposition. IHC for IL-2 indicates that LPR mice may have increased levels of IL-2 in their both their wounded and unwounded skin. In samples from lesions of SLE patients, IL-2-Receptor levels are higher compared with skin from healthy controls, which may reflect an increase in IL-2 signaling. L929 fibroblast cells in culture demonstrate impaired survival in response to IL-2 treatment. Thus, excess IL-2 in SLE might cause increased or premature fibroblast death and an inappropriate fibrotic response, which could lead to impaired healing and fibrosis in SLE patients.

Conclusions: Increased IL-2 signaling in the skin in SLE may be involved in the impaired wound healing and the pathogenesis of the fibrotic lesions in this disease.

Future directions: To further understand IL-2 signaling in the skin of SLE-model mice, IHC for IL-2-Receptor components will be performed. Furthermore, the downstream signaling pathways (Janus kinases, signal transducers and activators of transcription, and Src) will be explored as possible mediators of these affects.

NCBI GEO Reference: Jabbari A, Suárez-Fariñas M, Fuentes-Duculan J, Gonzalez J et al. Dominant Th1 and minimal Th17 skewing in discoid lupus revealed by transcriptomic comparison with psoriasis. J Invest Dermatol 2014 Jan;134(1):87-95. PMID: 23771123

INFLAMMATION

IN1 Effects of 1,2,3,4,6 Penta-O-Galloyl-β-D-Glucose on the Inflammatory Cytokines Release from Activated BV-2 Microglial Cells

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Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting more than 5 million Americans. In neurodegenerative diseases, inflammation and the activation of glial cells by a variety of factors appear to have an important role in pathways leading to neuronal cell death. Activated microglia cells strongly produce inflammatory mediators such as macrophage inflammatory proteins, monocyte chemoattractant proteins, and proinflammatory cytokines. Therefore, suppressing the expression of these inflammatory mediators may decrease the incidence of or the progression of AD. The compound 1,2,3,4,6 Penta-O-Galloyl-β-D-Glucose (PGG), a naturally occurring polyphenolic compound highly enriched in some medicinal herbs such as *Rhus Chinensis Mill*, has a potent anti-inflammatory effect through the inhibition of many cytokines in different experimental models. In the present study, we investigated the anti-inflammatory effect of PGG as an agent in LPS/IFNγ activated BV2 microglia cells and its potential for the treatment of neuroinflammatory/neurodegenerative diseases. Mouse cytokine antibody arrays were used to assess the effect of PGG in the release of proinflammatory cytokines, and ELISA experiments were performed to validate the results from the arrays. PGG signaling pathway was evaluated with NF-κB and MAP Kinase PCR arrays. The results obtained from the cytokine arrays and ELISA assays showed that PGG decreased the expression of monocyte chemotactic protein-5 (MCP-5) and matrix metalloproteinase 9 (MMP-9). Both of these cytokines are upregulated during the inflammatory process and neurodegeneration. PGG also exhibited an effect in the protein expression level of the signaling proteins involved in the NF-κB and MAP Kinase pathways. Therefore, PGG modulatory effects on the release of MCP-5 and MMP-9 cytokines may provide a novel therapeutic agent for the management of microglia-mediated neurodegenerative disorders.

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IN2 Identifying IQGAP1 Domains Essential for Leukocyte Transmigration

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In response to an inflammatory stimulus the local blood vessels dilate, their endothelium is activated by cytokines and chemokines and circulating leukocytes are recruited to move out of blood vessels into the areas of tissue damage. The majority of leukocytes undergo transendothelial migration (TEM) at endothelial cell junctions by use of a unique membrane system within the endothelial cells referred to as the lateral border recycling compartment (LBRC). IQ-domain GTPase-activating protein 1 (IQGAP1) was identified as an LBRC-associated protein and subsequently found to be essential for TEM (See abstract IN3 by Sullivan, et al.). IQGAP1 is a scaffolding protein comprised of six major interaction domains. It has been reported to interact productively with over 135 cellular proteins. In endothelial cells IQGAP1 localizes to cell borders and knocking down its expression prevents TEM. However, it is not known which of the many proteins it is known to interact with carry out its role in TEM.

To understand the critical role it plays in TEM, we knocked down IQGAP1 in endothelial cells and re-expressed truncated forms of IQGAP1 with specific regions deleted to identify the regions (and thereby the potential binding partners) that are critical for TEM. We show that the actin-binding domain and the calmodulin-binding isoleucine-glutamine (IQ) domain of IQGAP1 are critical for its function during TEM.

Human endothelial cells in which >80% of endogenous IQGAP1 was depleted by shRNA did not support TEM; re-expression of wild-type IQGAP1 restored TEM to control levels. Re-expression of IQGAP1 with a GFP-tagged construct after knockdown also showed IQGAP1 enrichment around migrating leukocytes. When IQGAP1 truncation mutants lacking the N-terminal actin-binding domain were re-expressed they did not localize to endothelial borders and did not support TEM. However, re-expression of truncated IQGAP1 constructs containing the actin-binding domain and the IQ domain localized to cell borders and supported TEM. Constructs containing the actin-binding domain but lacking the IQ domain localized to the cell borders but did not restore TEM.

After identifying the IQ domain as having a critical role in TEM, we generated specific point mutations in this domain within the full-length construct. In particular, the isoleucine (I)-glutamine (Q) motif after which the IQ domain is named has been identified in the literature as having an essential role in IQGAP1 function and we are currently testing whether

they are also important in TEM. Using these constructs in future studies will help identify the role of the IQ domain in TEM and its interacting partner(s). Identifying the binding partners of IQGAP1 that are critical for inflammation will provide clues to its mechanism of action and potential therapeutic targets for anti-inflammatory therapy.

IN3 The Role of IQGAP1 in Transendothelial Migration: From *In Vitro* Identification to *In Vivo* Validation

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Although inflammation is the body's routine response to tissue damage, the clinical outcomes, be they beneficial or detrimental, of this critical process are rooted in the types and functions of the leukocytes that are recruited to site of insult. It therefore follows that the regulation of leukocyte recruitment represents an acutely tractable target for therapeutic intervention. In order to reach the target tissue, leukocytes undergo a carefully choreographed series of sequential intercellular interactions with the endothelium that culminate in transendothelial migration (TEM). As the leukocyte prepares to traverse the endothelial cell layer requisite protein-protein interactions between the two cells signals the recruitment of the endothelial cell sub-junctional Lateral Border Recycling Compartment (LBRC). Several of the endothelial proteins crucial for this process reside in the LBRC and its function in TEM is required for efficient leukocyte efflux.

To identify additional proteins that are involved in LBRC function in an unbiased approach, we adapted a biochemical purification method known as the DAB density shift to specifically label and recover fractions enriched the LBRC proteins. Through this process we identified IQ domain containing GTPase Activating Protein 1 (IQGAP1) and vimentin as previously unknown LBRC associated proteins. IQGAP1 is a cytoplasmic multidomain scaffolding protein that has been shown in other systems to integrate and propagate complex signaling pathways. Genetic ablation of IQGAP1 in endothelial cell cultures using shRNA transfection substantially reduced their ability to support efficient monocyte TEM. Re-expression of full-length IQGAP1 rescued this defect, confirming its essential function in this process. Interestingly, re-expression of IQGAP1 constructs that lack the actin-binding or IQ-motif domains were not able to rescue the TEM defect (see abstract IN2 by Dalal *et al*.) shRNA mediated reduction of IQGAP1 expression did not affect the physical amount of LBRC membrane that could be isolated from endothelial cells suggesting instead that IQGAP1 plays a role in some signaling aspect or other function of the LBRC.

To extend these studies *in vivo* we created bone marrow chimeras (wild type leukocytes and IQGAP1 knockout endothelial cells) and investigated the role of endothelial cell IQGAP1 in inflammation using two separate mouse models. In both croton oil dermatitis and response to IL-1β in the cremaster muscle, visualized by 4D intravital microscopy, the ability of neutrophils to extravasate across the endothelium into inflamed tissues was significantly inhibited. These findings represent the first demonstration of a critical role for endothelial cell IQGAP1 in inflammation *in vivo*.

IN4 The Immunomodulatory Properties of Adipose Mesenchymal Stem Cells are Strengthened by Treatment With Osteoarthritic Synovial Fluid.

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Introduction: Recently, adipose mesenchymal stem cells' ability (AMSCs) to secrete several factors with various functions has been shown. Their local or systemic injection has been used to stimulate tissue repair in different clinical conditions, including joint inflammatory diseases. It has been reported that local injection of AMSCs in osteoarthritic joints improves function by inhibiting osteophyte formation and reducing cartilage degradation. AMSCs seem to promote tissue repair mainly by paracrine activity and their anti-inflammatory properties have been linked to their immunosuppressive ability. Here, we report data showing the immunosuppressive ability of AMSCs treated with synovial fluid (SF) collected from osteoarthritis joints.

Methods: AMSCs were cultured for 48h in the presence of SF (1:2 with medium) derived from 5 patients with gonarthrosis. Proliferation and viability were measured by trypan blue exclusion and expression of surface markers was evaluated by flow cytometry. AMSCs were than cultured in the presence of SF (1:2 with medium) for 6h, then medium was harvested after 48h and analysed by a 40 cytokines/chemokines/GFs Multiplex ELISA. CD14+ monocytes, isolated from blood donor PBMCs, were cultured with GM-CSF, supposed to induce M1 phenotype, and in three different conditions: 1) conditioned medium of unstimulated AMSCs; 2) conditioned medium of AMSCs pre-treated with 50% SF; 3) 50% SF for 7 days to study the influence of such treatments on M1 phenotype. After treatment, macrophages were characterized for the expression of CD80 (M1 expression marker) or CD163 (M2 expression marker) by flow cytometry.

Results: When AMSCs were incubated with osteoarthritic SF, important morphological changes were induced. Specifically, an elongated and irregular shape and an increased proliferation rate (about 1.5 fold) were observed, whereas viability seemed not to be affected. The SF treatment increased the expression of specific staminal markers such as CD105, CD73 and ALP and stimulated the release of several chemokines involved in leukocytes recruitment (CX3CL1, CXCL6 and CXCL1). Finally, only the conditioned medium of SF-treated AMSC was able to significantly reverse the M1 phenotype of macrophages (19.5%±8.6% in control versus 46%±6.3% CD163 positive cells in AMSC SF-treated cultures), indicating a polarization towards the M2 phenotype.

Conclusions: The pro-inflammatory effect of osteoarthritic SF can activate AMSCs and induce immunosuppressive and anti-inflammatory properties through the release of soluble factors which shift macrophages from M1 to M2 phenotype. This suggests that the inflammatory microenvironment may play a role in controlling the activation and function of AMSCs, which could be particularly important in future regenerative medicine applications.

LIVER PATHOBIOLOGY

L1 IGF-1 Stability is Decreased During Acute Liver Failure in Mice Due to a Suppression of Acid Labile Subunit Expression Resulting **from Aberrant TGFβ1 Signaling**

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Acute liver failure can arise from hepatotoxic insults with acetaminophen (APAP) being the most common cause of acute liver failure in the United States. Transforming growth factor beta 1 (TGFβ1) is involved in liver fibrogenesis and is elevated in the serum following liver injury. Insulin-like growth factor 1 (IGF-1) is a hepatoprotective peptide that that has been shown to be suppressed by TGFβ1 signaling in other organs. Degradation of IGF-1 is reduced when bound by acid labile subunit (ALS). Therefore, we hypothesize that TGFβ1 suppresses ALS expression, leading to increased IGF-1 degradation and a subsequent worsening of APAP-induced hepatotoxicity.

Methods: Male C57Bl/6 mice or floxed TGFβ1 x albumin-cre ($TGFβ1^{fl/fl}$/Alb-Cre) mice were fasted for 12 hours prior to APAP injection and after 48 hours livers and serum were collected. In parallel, APAP-treated mice and saline-injected controls were pretreated with GW788388, a TGFβR1 antagonist, for 1 hour prior to APAP injection. In all groups, liver histology was assessed by hemotoxylin & eosin staining and liver damage determined via serum ALT and bilirubin measurement. TGFβ1, IGF-1 and ALS protein and mRNA expression were assessed by immunoblotting, immunohistochemistry, ELISA assays and/or qPCR. Formation of IGF-1/ALS protein complexes was measured by co-immunoprecipitation and immunoblotting. Nitrotyrosine, reactive nitrogen

species, reactive oxygen species, and glutathione were measured using commercially available kits.
Results: Mice injected with APAP had elevations of hepatic TGFβ1 and decreases in both IGF-1 and ALS expression. Mice treated with GW788388 or TGFβ1$^{fl/fl}$/Alb-Cre mice had less liver damage and improved liver function as assessed by hemotoxylin & eosin staining and ALT/bilirubin serum concentrations respectively. TGFβ1 expression was nearly absent in TGFβ1$^{fl/fl}$/Alb-Cre though was still highly expressed in GW788388-treated mice administered APAP. Both IGF-1 and ALS expression in GW788388-treated mice and TGFβ1$^{fl/fl}$/Alb-Cre mice were higher when compared to appropriate APAP-treated controls. The formation of IGF-1/ALS protein complexes was also increased in GW788388-treated mice and TGFβ1$^{fl/fl}$/Alb-Cre mice compared to appropriate APAP-treated controls. Nitrosative and oxidative stress, which were increased in APAP-treated C57Bl/6 mice, were reduced in GW788388-treated mice and TGFβ1$^{fl/fl}$/Alb-Cre mice.
Conclusion: APAP-induced liver injury is driven by a suppression of IGF-1/ALS due to hepatocyte TGFβ1 receptor-mediated signaling. These data demonstrate that the TGFβ1/IGF-1 signaling axis may act as a potential therapeutic target for the management of APAP-induced liver injury.

L2 Acetaminophen-induced Hepatoxicity in Mice is Driven by **TGFβ1 signaling**

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Acetaminophen (APAP)-induced hepatotoxicity is the leading cause of acute liver failure in the United States and Western Europe with mortality approaching 30%. Previous research by our lab has identified that transforming growth factor beta 1 (TGFβ1) is upregulated in a mouse model of hepatic encephalopathy due to acute liver failure and TGFβ1 contributes to the pathology of this syndrome. In order for TGFβ1 to induce its effects, it must signal through a heterotetramer receptor complex made up of TGFβ receptor 1 (TGFβR1) and TGFβ receptor 2 (TGFβR2), which leads to the phosphorylation of SMAD2 and SMAD3. At this time, little is known about the role of TGFβ1 during APAP-induced liver failure. Therefore, we hypothesize that TGFβ1 promotes hepatic damage and pathology associated with APAP-induced hepatotoxicity.
Methods: Male C57Bl/6 mice were fasted for 12 hours prior to injection of APAP and after 48 hours livers and serum were collected. In parallel, APAP-treated mice and saline-injected controls were pretreated with GW788388, a TGFβR1 antagonist, for 1 hour prior to APAP injection. In all groups, liver histology was assessed by hemotoxylin & eosin staining and liver function was determined via ALT and bilirubin measurement. TGFβ1, TGFβR1, TGFβR2 and SMAD2/3 expression were assessed by immunoblotting, immunohistochemistry and/or qPCR. Nitrotyrosine, reactive nitrogen species, reactive oxygen species, and glutathione were measured using commercially available kits.
Results: Mice injected with APAP had elevations of hepatic and circulating TGFβ1 as well as an increase in the phosphorylation of SMAD2/3 with no change in hepatocyte TGFβR1 or TGFβR2 expression. APAP-treated mice that were pretreated with GW788388 had elevated TGFβ1 but had reduced phosphorylation of SMAD2/3. Pretreatment with GW788388 was found to reduce APAP-induced hepatic damage and led to improved liver function as seen by a reduction in both ALT and bilirubin levels. Oxidative and nitrosative stress that was induced in APAP-treated mice was reduced in APAP-treated mice that were pretreated with GW788388. Finally, the increased oxidation state of glutathione and increased nitrotyrosine found in liver homogenates from APAP-treated mice were alleviated by pretreatment with GW788388.
Conclusion: Elevated TGFβ1 following APAP-induced liver failure contributes to activation of pSMAD2/3, which generates increased liver damage and nitrosative/oxidative stress. These deleterious effects can be reversed by inhibiting TGFβ1 signaling via GW788388, indicating that TGFβ-receptor mediated signaling could be a potential therapeutic target to help manage APAP-induced hepatotoxic liver injury.

L3 Liver-**Derived TGFβ1 Contributes to the Development of** Hepatic Encephalopathy due to Acute Liver Failure

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Hepatic encephalopathy (HE) is a neurological complication that arises due to loss of liver function and is associated with increased blood-brain barrier permeability, neuroinflammation and subsequent onset of cognitive decline. We have previously shown that there is increased expression and secretion of transforming growth factor β (TGFβ1) from hepatocytes in a mouse model of acute liver failure and this increase in circulating TGFβ1 contributes to the opening of the blood brain barrier and resulting neurological complications. The aims of this study were to definitively demonstrate that liver-derived TGFβ enters the brain and contribute to the pathogenesis of hepatic encephalopathy.
Methods: Liver-specific TGFβ1 knockout mice (TGFβ1/Alb-Cre) and neuron-specific TGFβR2 knockout mice (TGFβR2/Thy1-Cre) were injected with azoxymethane (AOM; 100 mg/kg ip). In parallel, C57Bl/6 mice were treated with anti-TGFb antibody (1 mg/kg; iv) 1 hour prior to AOM injection. Cognitive impairment was monitored by assessment of reflex responses and ataxia and livers, serum, brain tissue (for molecular analyses and edema assessments) and whole brains were collected. H&E staining and serum ALT and bilirubin levels were performed to assess liver damage. TGFβ1 and phosphor/total SMAD2/3 expression was assessed by immunoblotting, immunohistochemistry, EIA and/or RT-PCR. Microglia activation was assessed by morphometric analysis of Iba-1 immunoreactivity.
Results: TGFβ1 mRNA and protein expression were increased in liver and serum after AOM injection to wildtype mice. There was a concomitant increase in TGFβ1 protein levels in the frontal cortex after AOM injection without a parallel increase in mRNA expression. The AOM-induced neurological decline and neuroinflammation was delayed in TGFβ1/Alb-Cre mice but was associated with a decrease in liver damage suggesting that the delay in the development of HE maybe as a result of reduced underlying liver damage. However, pretreatment with neutralizing TGFβ1 antibody, which reduced TGFβ1 in the serum and cortex, delayed the onset of HE symptoms without protecting against the underlying liver damage. Furthermore, blocking TGFβR2 specifically in the brain also delayed the onset of HE symptoms without protecting against the underlying liver damage. The expression of phosphor SMAD2/3 and microglia activation were decreased in all 3 groups of mice compared to their appropriate controls.
Conclusions: Taken together, these data suggest that liver-derived TGFβ1 is responsible for the aberrant TGFβR2 signaling in neurons that contribute to the neurological complications associated with acute liver failure.

L4 GnRH Increases Liver Fibrosis in the Mdr2-/- mouse Model of Primary Sclerosing Cholangitis by Increased Epithelial-mesenchymal Transition (EMT) in Small Cholangiocytes and Enhanced Senescence in Large Cholangiocytes

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Background: Biliary damage and liver fibrosis are hallmarks of human cholestatic liver diseases, such as primary sclerosing cholangitis (PSC). PSC is characterized by biliary injury that contributes to extensive liver fibrosis. The intrahepatic biliary tree is formed by large, senescent cholangiocytes and small less senescent cholangiocytes. Gonadotropin-releasing hormone (GnRH) is a trophic peptide hormone (synthesized from hypothalamic neurons and cholangiocytes) that increases biliary proliferation and liver fibrosis by interaction with GnRH receptor 1 (GnRHR1) in normal and cholestatic models. Studies have shown that epithelial mesenchymal transition (EMT) and biliary senescence contributes to liver fibrosis in cholangiopathies. In this study, we aimed to demonstrate that the axis GnRH/GnRHR1 contributes to liver fibrosis via

differential changes in EMT/senescence in small and large cholangiocytes, respectively.
Methods*: In vivo* studies were performed in (i) small and large cholangiocytes isolated by laser capture microscopy (LCM) from normal FVB/NJ (WT) mice, treated with saline or GnRH (250 ng/Kg BW) for 1 wk, and Mdr2$^{-/-}$ mice; and (ii) early and late human male PSC patient samples. We evaluated the mRNA expression of (a) GnRHR1 and the expression of markers for (b) EMT Vimentin, N-cadherin (Cdh2), S100a4; (c) epithelial phenotypes, E-cadherin (Cdh1), fibronectin 1 (Fn-1); (d) markers of senescence, CCL2, p16, p21 and p18; and (e) fibrosis markers, α-SMA, collagen-1, Fn-1 and TIMP-1 by qPCR; and (iii) serum GnRH levels in early and late human male PSC patients by ELISA. *In vitro,* small and large murine cholangiocytes were treated with GnRH (100 nmol/L, 24 hr) before evaluating the expression of GnRHR1, EMT, epithelial, senescence and fibrosis markers by qPCR.
Results: There was increased GnRHR1, EMT and fibrosis (but not senescence) marker expression in small cholangiocytes, whereas senescence markers were increased in large cholangiocytes from normal WT mice treated with GnRH and Mdr2$^{-/-}$ mice compared to the control WT group. GnRHR1, EMT, epithelial, senescence and fibrosis markers in total liver samples from PSC early and late stage male patients were increased compared to control. Elevated GnRH serum levels were seen in WT mice treated with GnRH and in Mdr2$^{-/-}$ mice compared to normal WT, and in PSC early and late stage male patients compared to control. *In vitro*, GnRH increased fibrosis through increased EMT in small cholangiocytes and senescence in large cholangiocytes.
Conclusions: GnRH contributes to liver fibrosis by differentially increasing EMT in small cholangiocytes and senescence in large cholangiocytes. Modulation of the GnRH/GnRHR1 axis in cholangiocytes may be important for the management of PSC.

L5 Role of Sumoylation in Aldehyde Dehydrogenase 2 Function During Alcoholic Liver Disease and Liver Cancer Development

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Background: Chronic alcohol abuse causes liver disease that progresses from steatosis through stages of steatohepatitis, fibrosis, cirrhosis and eventually hepatic failure. Acetaldehyde, a major toxic ethanol metabolite, is mutagenic and carcinogenic, playing an important role in the pathogenesis of alcoholic liver disease (ALD) inducing production of reactive oxygen species (ROS) and apoptosis. ALDH2 is the major enzyme that metabolizes acetaldehyde produced from alcohol metabolism and it is well know to be correlated with the high risk for hepatocellular cancinoma (HCC) development. Sumoylation is a posttranslational modification able to modify the activity, localization, and expression of the target protein by a covalent bind where small ubiquitin modifier (SUMO) is a tag. Increased ROS production enhances the global protein SUMO conjugation profile. We previously demonstrated that the level of Ubiquitin-conjugation enzyme 9 (Ubc9), a sole E2-conjugating enzyme essential for sumoylation, is induced in the livers of intragastric ethanol-infusion (EI) treated mice. Our aim was to examine whether the dysregulated sumoylation could influence the ethanol-induced ALDH2 function in both ALD and HCC, elucidating the molecular mechanism(s).
Methods: Studies were done using *in vivo* binge ethanol-fed mice, primary mouse hepatocytes and HepG2 cells (HCC cell line) . Sumoylation protein profiling and expression were measured by Mass Spectrometry and Western blot analyses, respectively. ALDH2 activity was analyzed using a commercial kit.
Results: We found that ALDH2 is sumoylated in binge mice livers analyzing the peptide-sequencing data from sample purified by SUMO immunoaffinity columns. In addition, we found that sumoylation machinery gene group (Ubc9, SUMO-1, SUMO-2 and SUMO-3) and ALDH2 are increased at both mRNA and protein levels in the livers of binge mice, ethanol-treated hepatocytes and in HepG2 cells. Surprisingly, we found that only SUMO-3 silencing prevents ethanol-mediated induction in ALDH2 protein level. ALDH2 activity assay showed that SUMO-3 inhibits ALDH2 enzymatic activity in primary mouse hepatocytes and HepG2 cells. Finally, we confirmed the peptide-sequencing data of sumoylated ALDH2 using in vivo binge mice livers finding increased level of SUMO1/2/3-ALDH2 complex formation following ethanol treatment.
Conclusions: We report for the first time that ethanol-mediated sumoylation increased ALDH2 protein level in livers of binge mice. Moreover, we demonstrated that sumoylation prevents the enzymatic activity of ALDH2 lowering its protein stability that could induce the ROS accumulation. This novel finding thereby opens a new area of investigation examining the importance of ALDH2 sumoylation in alcoholic liver disease and in HCC suggesting its potential role in the therapeutic approach.

L6 Role of Sumoylated SOD2 in Alcoholic Liver Disease and Liver Cancer

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Background: Manganese superoxide dismutase (MnSOD, SOD2) is the most important mitochondrial enzyme involved in the defense against reactive oxygen species (ROS) and it is well demonstrated to be a key protein in hepatocellular carcinoma (HCC) progression and in other several cancers development as kidney and colon adenocarcinoma. Alcohol abuse is one of the main causes of alcoholic liver disease (ALD) and represents the major risk factor of HCC development. Alcohol abuse generates a high level of ROS as a consequence of increased enzymatic activity during the ethanol metabolism. SOD2 plays a crucial role in the build-up of tolerance to ethanol catalyzing the dismutation of $O_2^{\bullet-}$ in H_2O_2. SUMOylation is a post-translational modification that regulates a wide range of cellular processes involving the covalent attachment of a member of the SUMO (small ubiquitin-like modifier; SUMO-1, SUMO-2 and SUMO-3) family of proteins to lysine residues in specific target proteins via an enzymatic cascade. Increased ROS production enhances the global protein sumoylation profile. Our aim was to examine whether sumoylation could regulate SOD2 function in ALD and in HCC elucidating the molecular mechanism(s).
Methods: Chromatography by SUMO binding columns was performed to purify sumoylated proteins form total livers of binge ethanol fed mouse model. Primary mouse hepatocytes and HepG2 cells (HCC cell line) were used to perform *in vitro* experiments. Protein and mRNA levels were measured using Western blotting and RT-PCR, respectively. SOD2 activity was measured by tetrazolium salt commercial kit.
Results: Using sumoylated purified-proteins, we found that SOD2 is sumoylated in binge ethanol fed mouse liver. *In vitro* experiments showed a different trend: Ethanol treatment lowers SOD2 protein and mRNA levels in primary mouse hepatocytes and HepG2 cells. Interestingly, SUMO-3 silencing prevents ethanol stress-damage increasing the SOD2 enzymatic activity raising protein stability in primary hepatocytes. In contrast, knockdown of SUMO-1 lowers SOD2 enzymatic activity and protein level indicating that SUMO-1 may play an important role in the oxidative stress prevention.
Conclusion: We report for the first time that ethanol increases sumoylation of SOD2. In addition, SUMO-1 and SUMO-3 control SOD2 protein stability and enzymatic activity in primary mouse hepatocytes and HepG2 cells. This new finding opens a new area of investigation examining the importance of SOD2 sumoylation in ALD and HCC, suggesting SOD2 as a potential therapeutic target to protect against induced-oxidative stress liver damage.

L7 Therapeutic Potential of H1 or H2 Histamine Receptor Antagonists on Hepatic Fibrosis, Liver Damage, and Mast Cell Migration in Mdr2$^{-/-}$ Mice and Human PSC

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Background: Primary sclerosing cholangitis (PSC) is a cholestatic disease that arises from damaged cholangiocytes, epithelial cells that line the biliary tree. The multi-drug resistant 2-gene knockout (Mdr2$^{-/-}$) mouse histopathologically mimics human PSC. Following bile duct ligation that

induces cholestatic injury, mast cells (MCs) increase in number and secrete histamine that increases biliary proliferation, intrahepatic ductal mass (IBDM), and hepatic fibrosis by interaction with the H1 and H2 histamine receptors (HRs). H1/H2 HR blockers like Claritin® and Zantac® are used to alleviate allergic reactions and heartburn, but their effect on cholangiopathies is unknown; therefore, we aimed to evaluate the effects of prolonged usage of H1 and H2 blockers on MC numbers, biliary proliferation, hepatic damage, and fibrosis in Mdr2$^{-/-}$ mice, and to measure MC numbers in human PSC.
Methods: Wild-type (WT) and Mdr2$^{-/-}$ mice were treated by osmotic minipumps with 0.9% NaCl, fexofenadine (H1HR antagonist, 10mg/kg BW/day) or ranitidine (H2HR antagonist, 10mg/kg BW/day) for 4 weeks. Liver blocks, serum, and isolated cholangiocytes were obtained. Cholangiocyte proliferation and IBDM were evaluated by immunohistochemistry for Ki-67 and CK-19, respectively. Fibrosis was detected by Sirius Red staining and by qPCR for the fibrotic markers, α-SMA, fibronectin, collagen type-1α, and TGF-β1. Serum HA levels were measured by EIA. Necrosis, inflammation, and lobular damage were assessed by H&E. MC markers were evaluated by qPCR for chymase, tryptase, c-Kit, and FCe receptor. *In vitro,* murine hepatic MCs were treated with NaCl, fexofenadine (10 mM), or ranitidine (25 mM) for 24 hrs before HA secretion and MC marker expression was measured. In human control and PSC patient samples, serum HA levels were measured by EIA and MC markers were evaluated by qPCR for chymase, tryptase, c-Kit, and FCe receptor and immunohistochemistry for chymase and tryptase.
Results: In Mdr2$^{-/-}$ mice treated with H1/H2 HR blockers there was a significant decrease in liver damage, IBDM, proliferation, liver fibrosis, and MC number when compared to saline treated Mdr2$^{-/-}$ mice. Histamine secretion was reduced in Mdr2$^{-/-}$ mice treated with antagonists compared to saline treated Mdr2$^{-/-}$ mice. No adverse effects on biliary proliferation, liver damage, fibrosis, or MC number were found in WT mice treated with HR blockers. *In vitro,* stimulation with H1 or H2 blockers reduced MC histamine release and MC marker expression. PSC patients show increased liver MC markers and HA serum levels.
Conclusion: Chronic usage of H1 or H2 blockers has no adverse effect on WT mice, but reduces liver damage, biliary proliferation, fibrosis, and MC number in Mdr2$^{-/-}$ mice. PSC patients show increased MC number and HA secretion, which may increase damage associated with this disease. H1 and H2 blockers may be a promising therapy for patients suffering from cholangiopathies such as PSC.

L8 Bile Acid-**Mediated FXR/SHP Signaling Suppresses LXRβ** Leading to Dysregulated Cholesterol Homeostasis in the Brain during AOM-induced Hepatic Encephalopathy

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During acute liver failure (ALF), the enterohepatic circulation becomes disrupted leading to bile acid accumulation in serum. Previous research by our lab has shown that bile acids induce permeability of the blood-brain barrier and contribute to the development of hepatic encephalopathy (HE) following ALF. This occurs via activation of farnesoid X receptor (FXR), which, in turn, upregulates transcription of small heterodimer partner (SHP) in neurons. The events downstream of FXR activation contributing to HE are unknown. However, FXR activation is known to decrease the expression of liver X receptor beta (LXRβ). Highly expressed in neurons in the brain, LXRβ has a role in regulation of Cyp46A1, an enzyme responsible for converting cholesterol to 24(S)-hydroxycholesterol—the main mechanism of cholesterol clearance in the brain. We hypothesize that increased levels of cholesterol in the brain during ALF and HE result from bile acid-mediated activation of the FXR/SHP pathway and LXRβ suppression leading to a dysregulation of cholesterol clearance.
Methods: C57Bl/6 mice or cytochrome p450 7A1 knockout (Cyp7A1$^{-/-}$) mice were injected with azoxymethane (AOM) to induce ALF and HE. In parallel, C57Bl/6 mice were given an intracerebroventricular infusion of FXR Vivo-morpholino sequences to reduce FXR protein expression prior to AOM injection. Additionally, a 5-day pravastatin gavage in C57Bl/6 mice was performed before AOM injection. Cognitive impairment was monitored by assessment of reflex responses and ataxia. Primary neurons were isolated from P1 C57Bl/6 mouse pups and treated with deoxycholic acid (DCA) and/or guggulsterone (an FXR inhibitor). Liver histology was assessed with hematoxylin and eosin staining. Liver function was assessed by measuring serum AST, ALT and bilirubin levels. Cyp46A1, SHP, and LXRβ expression were assessed by immunoblotting and qPCR. Brain and serum levels of cholesterol and 24(S)-hydroxycholesterol were measured using commercially available kits.
Results: AOM-treated mice had elevated cholesterol and decreased 24(S)-hydroxycholesterol in the brain, compared to saline-injected controls. This result was not observed in AOM-treated Cyp7A1$^{-/-}$ mice. Cyp46A1 was downregulated in AOM-treated mice but not in AOM-treated Cyp7A1$^{-/-}$ mice. DCA-treated primary neurons had reduced Cyp46A1 protein and mRNA expression, and this downregulation was inhibited when co-treated with guggulsterone. In addition, AOM-treated FXR Vivo-morpholino sequence-infused mice had no change in Cyp46A1 expression compared to mismatched-morpholino infused controls. SHP was upregulated while LXRβ was downregulated in the brain following AOM injection, and these effects were not observed in AOM-treated mice that were infused with FXR Vivo-morpholino sequences. AOM-treated mice gavaged with pravastatin showed decreased levels of AST, ALT, and bilirubin, reduced liver damage, lowered serum and brain cholesterol levels, and reduced cognitive impairment compared to control AOM-treated mice. Cholesterol concentrations in the brain of pravastatin-gavaged mice were decreased, while 24(S)-hydroxycholesterol concentrations were increased, compared to control mice.
Conclusion: During AOM-induced ALF and HE, increased cholesterol levels in the brain are mediated by bile acid activation of FXR and SHP, which together inhibit Cyp46A1 expression via the inhibition of LXRβ. FXR/SHP-mediated signaling may be a possible therapeutic target for the management of HE through its interaction with proteins controlling cholesterol homeostasis.

L9 Knockdown of α7-Nicotinic Receptor Inhibits Biliary Proliferation and Hepatic Fibrosis during Extrahepatic Cholestasis

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In cholestatic liver diseases, cholangiocytes, through the products of their cellular activation, are implicated as the key link between bile duct injury and the subepithelial fibrosis that characterizes chronic hepatobiliary injury. We have previously shown that activation of the α7-nicotine receptor (nAChR) with nicotine stimulates biliary proliferation that was associated with increased profibrotic gene expression by cholangiocytes and deposition of collagen in portal areas in normal wild-type (WT) mice. We have also shown that mechanical stress, which occurs due to increased biliary pressure during extrahepatic cholestasis, induces biliary hyperplasia. The role of the α7-nAChR during extrahepatic cholestasis or mechanical stress has not been thoroughly explored. Thus, the AIM of our study was to determine the role of α7-nAChR in the regulation of biliary proliferation and hepatic fibrosis during extrahepatic cholestasis induced by bile duct ligation (BDL).
Methods: Studies were performed in normal and BDL (1 week) WT and α7-nAChR knockout (KO) mice. Intrahepatic bile duct mass (IBDM) and biliary proliferation was evaluated by immunohistochemistry for CK-19 and PCNA, respectively, in liver sections and by immunoblots in isolated cholangiocytes. Liver fibrosis was evaluated by Sirius red staining in liver sections and by *q*PCR for the profibrotic markers [collagen 1 (COL1A1), fibronectin (FN-1), and αSMA] in isolated cholangiocytes and total liver samples. *In vitro*, mouse primary cholangiocytes were plated on BioFlex culture plates and static equiaxial strain was applied to the cells for 24 hr in the presence and absence of shRNA to knockdown α7-nAChR and the α7-nAChR antagonist methyllycaconitine (1.4 nM). Proliferation was evaluated by immunoblots for PCNA and the expression of COL1A1, FN-1 and aSMA by qPCR.
Results: *In vivo*, there was a significant reduction in biliary proliferation (PCNA) and IBDM (CK-19) in BDL α7-nAChR KO mice compared to BDL WT. In addition to a reduction in proliferation, there was also a decrease in hepatic fibrosis by Sirius red staining and a significant reduction in

COL1A1, FN-1 and αSMA gene expression. *In vitro*, knockdown or inhibition of α7-nAChR in mouse cholangiocytes prevented mechanical stress-induced biliary proliferation. Knockdown or inhibition of α7-nAChR also inhibited mechanical stress-induced COL1A1, FN-1 and αSMA gene expression.
Conclusion: The knockout of α7-nAChR inhibits BDL induced proliferation and hepatic fibrosis. Modulation of the α7-nAChR axis during extrahepatic cholestasis may represent a novel therapeutic approach for cholestatic liver diseases.

L10 Biosafety Assessment of Petroleum Ether Oil of *Aframomum melegueta* K. Schum in Wistar Rats

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Objectives: The study was aimed at evaluating the biosafety of reported insecticidal potency of *Aframomum melegueta* petroleum extract (AMPE) used on stored products using wistar rats as a model.
Methodology: *Aframomum melegueta* seeds were extracted using soxhlet extractor and the filtrate later concentrated using the rotary evaporator to obtain *A. melegueta* petroleum ether extract (AMPE). Twenty four rats were divided into four groups labeled I, II, III and IV (six rats per group). Group I served as the control group and were administered orally with dimethyl sulfoxide (DMSO) and normal saline, Group II were administered orally with 500mg/kg of AMPE, Group III were administered orally with1000mg/kg of AMPE while Group IV were administered orally with 2000mg/kg of AMPE all dissolved in DMSO and normal saline. Animals were weighed, anesthetized and blood samples were collected at the end 5 days of oral administration. Blood samples were collected by cardiac puncture for some biochemical parameters such as Total proteins, bilirubin, cholesterol, uric acid, creatinine, Urea and Uric acid activities as well as serum, AST and ALT were determined.
Results: At the end of this research work, it was discovered that at doses higher than 500mg/kg there were enzymatic leakages from the organs (kidney and liver) into the serum, compared to the control group.
Conclusion: The study revealed that it is advisable to use minimal dosages for the management of insect pest on products meant to be consumed by human, to prevent damage to vital organs as observed in the results at higher doses while higher doses should be for seeds meant for propagation.

L11 The Effect of mTOR Inhibition on the Proteome of Persistent Focal Lesions in a Rat Model of Hepatocarcinogenesis

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Hepatocellular carcinoma (HCC), the most common type of liver cancer, has a very high rate of recurrence and a five-year survival rate of less than 5%. There are currently no approved chemopreventive therapies for HCC. Previous studies in our laboratory have shown that the mechanistic target of rapamycin (mTOR), a nutrient-sensing serine/threonine protein kinase that regulates cell cycle progression, protein synthesis, and ribosomal biogenesis, is critical to the development and progression of preneoplastic foci in the rodent model of experimental hepatic carcinogenesis. In addition, microarray analyses indicated that short-term inhibition of mTORC1 signaling early in the process of hepatic carcinogenesis results in marked, persistent changes in the genetic signature such that the resultant focal lesions more closely resemble normal liver.
The aim of this study was to characterize the effect of mTOR inhibition on the proteome of persistent preneoplastic focal lesions in this model. We hypothesized that the changes in gene expression would be translated to the proteome resulting in greater similarity with the normal liver proteome. To test this hypothesis, hepatic carcinogenesis was induced using the resistant-hepatocyte model. Rats were administered rapamycin via a 9.45-mg, 21-day, slow release pellet, starting at the time of partial hepatectomy. Control animals received a placebo pellet. Animals were euthanized 21 days after cessation of rapamycin treatment. Persistent focal lesions from formalin-fixed-paraffin-embedded tissues from rapamycin and placebo treated rats were dissected by laser capture microdissection. Peptides profiles were analyzed by liquid chromatography-mass spectrometry. MS/MS spectra results were searched against the UniProt database and p-values were calculated from five replicates. Statistically significant peptides were determined using two-tailed unpaired Student's t-tests.
Our results identified 13,731 unique peptides across all samples with high degree of overlap (≥ 77%) between biological replicates. In addition, we showed that there were more significant (FDR<0.05) peptides in the placebo:normal liver comparison than there were in the rapamycin:normal liver comparison. Peptides were selected for pathway analysis using a FDR q-value of <0.05, and a fold change beyond a calculated inflection point. Pathway analyses showed similar significant pathways comparing rapamycin or placebo to normal liver. Notably, NRF2-mediated oxidative stress response, xenobiotic metabolism signaling and LPS/IL-1 mediated inhibition of RXR function were identified and significant in both comparisons.
In conclusion, this study explores the methodology allowing for proteome analysis from limited amount of formalin-fixed, laser-dissected tissue, with large scale, accurate qualitative and quantitative results and provides insights into the dynamics between gene profiling and proteome analyses. We showed that at the level of the proteome, the rapamycin focal lesions are not dissimilar to the placebo lesions. Future studies will focus on profiling the phosphoproteome to identify other signaling pathways involved in the growth and development of hepatocellular carcinoma.

L12 Glypican 3 (GPC3)-CD81 Axis Regulates Spleen Tyrosine Kinase(Syk)-Ezrin/Merlin Mediated Hippo Pathway via Crosstalking with HGF/c-Met Axis in Hepatocytes and Hepatocellular Carcinoma (HCC)

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Investigations of pathways in normal and abnormal circumstances are essential to develop better targeted therapies. GPC3 is over-expressed in HCC, and associated with inhibition of hepatocyte growth and liver regeneration. We have also shown that GPC3 binds to the membrane tetraspanin CD81, which is one of the portals of entry of hepatitis C virus (HCV). Studies elsewhere have shown that activation of CD81 is associated with phosphorylation of Ezrin, which regulates nuclear levels of yes-associated protein (Yap). It also has been shown that HGF/Met axis phosphorylate Ezrin as well by unknown mechanisms. Expression of Yap in the nucleus enhances hepatocyte growth. Mice over-expressing GPC3 have decreased levels of nuclear Yap.
We have demonstrated that GPC3 binds to CD81; the purpose of this study is to explore the role of GPC3 in regulation of Yap via CD81 and the possible mechanisms. We found that treatment of primary rat hepatocytes with CD81 agonist antibody or HGF led to higher levels of phosphorylated Ezrin and lower Hippo activity in a Syk dependent manner. Partial hepatectomy (PHx) was associated with decreased binding of GPC3 to CD81 during early liver regeneration, CD81 activated Ezrin and downregulated Hippo activity *in vivo*. Transgenic Met knock out mice showed more Hippo activity and less Yap expression at baseline level, and abrogated upregulated Yap expression, which was observed in wild-type mice after 2d PHx. Rat hepatoma cell lines JM1 and JM2 had dramatically down regulated CD81 expression and Hippo activity. Regain of CD81 expression in JM2 rat hepatoma cells upregulated Hippo activity while downregulated Ezrin activity. Human HCC tissue array revealed absence of CD81 in most HCCs but present in all the normal tissue controls, while GPC3 was present in most HCCs and all the normal tissue controls. CD81 expression could be detected in membrane and organelle fraction in human hepatoma cell lines HepG2, Huh7 and Hep3B, but immunoprecipitation experiment suggests that CD81 from the latter two human hepatoma cell lines has lost association with GPC3. Treatment with HCV envelope protein E2 led to decrease in nuclear Yap in HepaRG cells and primary human hepatocytes.

Conclusions: Both Met-phosphoEzrin and CD81-phosphoEzrin signaling enhances nuclear Yap. These pathways in normal hepatocytes are inhibited by GPC3. In human hepatocytes, GPC3 and the E2 protein of HCV also inhibit the CD81-phosphoEzrin effect and cause a decrease in nuclear Yap. Since most HCC lack expression of CD81, they are likely to escape from antagonistic modulation of over-expressed GPC3 and maintain high levels of Yap. The latter controls expression of GPC3, and thus may be the reason why most HCC express high levels of GPC3. HCC may also have a growth advantage over normal hepatocytes in HCV infection by missing CD81, thus being able to undergo clonal expansion, facilitating HCC early development and growth. Persistent HCV infection strengthens this expansion process and contributes to carcinogenesis by E2 protein interacting with CD81. The different distribution pattern of HGF/Met and GPC3/CD81 between normal hepatocytes and HCC cells provides the chance to develop precise anti-HCC therapies.

L13 Platelet-derived Growth Factor Receptor Alpha Contributes to Hepatic Stellate Cell Migration through mTOR and Focal Adhesion Kinase Activation *In Vitro*

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Platelet-derived growth factor receptor α (PDGFRα) is a tyrosine kinase receptor that plays a role in cell survival, proliferation, and differentiation, and is involved in liver development, regeneration and chronic liver injury states such as hepatic fibrosis and cirrhosis. Hepatic stellate cells (HSCs) are the primary mediators of hepatic fibrosis through their activation from a quiescent state, partially in response to the presence of pro-fibrotic growth factors. One of the key characteristics of this activation is an increase in HSC motility, which is believed to facilitate the migration of activated HSCs to sites of liver injury. In this study we investigate the role of PDGFRα on cell migration in primary human hepatic stellate cells (HHSteCs) using the PDGFRα –specific antagonist monoclonal antibody olaratumab (IMC-3G3). Through transwell migration assays we show that olaratumab reduces cell migration in HHSteCs. Detection of PDGFRα using phospho-specific antibodies show that olaratumab attenuates the activation of this receptor in response to PDGF-BB – a key ligand in hepatic fibrosis known to induce HSC motility. Furthermore, protein phosphorylation studies have identified a reduction in the activation of both mechanistic target of rapamycin (mTOR) and focal adhesion kinase (FAK) following treatment with olaratumab, suggesting that PDGFRα signaling may play a role in focal adhesion formation during cell migration through these key signaling kinases. These findings support a novel contribution of PDGFRα to HSC migration and provide evidence for its importance in chronic liver injury and hepatic fibrosis.

L14 **Simultaneous Ablation of β and γ**-Catenin Contributes to Loss of Blood Bile Barrier and Progressive Familial Intrahepatic Cholestasis Disease Like Phenotype

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Loss of blood bile barrier (BBlB) is central to liver cholestasis (reduction or stoppage of bile flow) in children as well as adults. However, the cellular and molecular mechanism that promulgates BBlB failure is incompletely understood and the current treatment for cholestasis are limited to surgery or liver transplantation suggesting that pre-emptive therapies based on the improved understanding of molecular pathways that enable BBlB failure are highly warranted.

Recent work in our lab has shown the involvement of β-catenin in cholestasis by potentially regulating the BBlB. β-catenin is a downstream signal transducer of the Wnt signalling pathway, which translocates to the nucleus following activation and regulates downstream target genes by binding to the cofactor T cell factor (TCF). β-catenin is also important in the maintenance of adherent junction and cell-cell adhesion. Through its association with E-cadherin, β-catenin stabilizes the actin cytoskeleton and promotes inter-cellular adhesion at cell-cell junction. Interestingly, we found that in the absence of β-catenin, another junctional protein γ-catenin is increased to compensate for the loss by maintaining the adherent junction structure. γ-Catenin is structurally and functionally highly similar to β-catenin and is considered as a nonidentical twin of the former. γ-catenin is also considered as a downstream effector of Wnt pathway and similar to β-catenin, upon pathway activation γ-catenin translocates to the nucleus to activate target gene expression albeit weakly. Interestingly, we found that similar to β-catenin knockout, loss of γ-catenin causes an increase in the level of β-catenin suggesting a compensatory mechanism between these two catenins in liver pathophysiology.

To unravel the mechanism that is essential for the maintenance of BBlB junctional stability, we made a double knockout of β- and γ-catenin (DKO) in the liver. Here we demonstrate the phenotype caused by knocking down both β- and γ-catenin. Our data shows that loss of both β and γ-catenin causes significantly higher serum bilirubin and alkaline phosphatase level. Interestingly, single knockout of β- or γ-catenin do not cause such a strong phenotype. We find that simultaneous ablation of β- and γ-catenin causes redistribution of several tight junctional proteins including claudins and occludin resulting in leaky tight junctions in DKO. Furthermore, by intravital imaging of liver we show that BBlB is lost in DKO mice causing mixing of blood with the bile. Remarkably, we also show that in a subset of patient's samples of progressive familial intrahepatic cholestasis (PFIC), which mimic the DKO mice phenotype, both β- and γ-catenin were lost. Taken together, these results highlight the importance of β- and γ-catenin in maintaining adherent junctional stability, distribution of tight junctional proteins thereby promoting their barrier function and finally the role in maintaining BBlB, which can prevent PFIC and other cholestatic liver diseases.

L15 Beta-catenin Independent Wnt Signaling Regulates Cholangiocyte Proliferation and Function During Cholestasis

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Wnt/β-catenin signaling has been implicated in a variety of liver pathologies, including atypical ductular proliferation during hepatobiliary injury. Previously, we have shown that liver-specific β-catenin knockout (KO) mice exhibited a markedly decreased ductular response after 2 weeks of 0.1% 3,5-diethoxycarbonyl-1,4-dihydro-collidine (DDC) diet. More recently, we identified upregulation of specific Wnt proteins in cholangiocytes during cholestatic liver injury. Additionally, mice lacking Wnt secretion from hepatocytes and cholangiocytes (Alb-cre Wls KO) showed fewer proliferating cholangiocytes and high mortality in response to DDC diet. Thus, we hypothesize Wnt induces proliferation of cholangiocytes, which may alleviate some of the complications caused by cholangiopathies. Through *in vitro* studies, we demonstrate that two of the Wnts increased during cholestasis, Wnt7B and Wnt10A, promote proliferation of cholangiocytes in an autocrine manner. These Wnts are specific for cholestasis, as wild-type (WT) mice show normal expression of Wnt7B and Wnt10A after choline-deficient, ethionine-supplemented diet, which induces hepatocyte injury and ductal hyperplasia unrelated to cholestasis. Intriguingly, proliferation and cell viability were unimpaired in a small cholangiocyte (sm-cc) cell line after β-catenin suppression *in vitro* under normal growth conditions. To confirm these findings, sm-cc cells were transfected with either Wnt7B or Wnt10A, in combination with either control or β-catenin siRNA. The absence of β-catenin did not inhibit the Wnt 7B- and 10A-induced increase in cholangiocyte proliferation. Further, in liver-specific Wnt co-receptor low-density lipoprotein related protein (LRP) 5/6 KO, which lack canonical Wnt/beta-catenin signaling, cholangiocyte proliferation was unaffected, suggesting a β-catenin independent role for Wnt signaling in this cell type. Finally, media from sm-cc cells transfected with either Wnt7B or Wnt10A was analyzed for cytokine production. Compared to control cells, Wnt7B and Wnt10A induced a significant increase in the expression of cytokines and growth factors such as IL-1A, IL-17A, and VEGF, while expression of anti-inflammatory IL-2 decreases in the transfected cells. Thus, β-catenin independent Wnt signaling promotes ductular reaction during cholestasis and may also alter the phenotypic response of proliferating cholangiocytes.

L16 Dysregulation of Genes Controlling Proliferation and Critical Biosynthetic Pathways Following Systemic Elimination of MET and EGFR Signaling in Regenerating Liver

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Receptor tyrosine kinases MET and EGFR are critically involved in initiation of liver regeneration. Other cytokines and signaling molecules also participate in the early part of the process. Regeneration employs effective redundancy schemes to compensate for the missing signals. Elimination of any single extracellular signaling pathway only delays but does not abolish the process. Our present study, however, shows that combined systemic elimination of MET and EGFR signaling (METKO+EGFRi mice) abolishes liver regeneration, prevents restoration of liver mass and leads to liver decompensation. METKO or simply EGFRi mice had distinct and signaling-specific alterations in Ser/Thr phosphorylation of mTOR, AKT, ERK1/2, PTEN, AMPKα etc. In the combined MET and EGFR signaling elimination of METKO+EGFRi mice, however, alterations dependent on either MET or EGFR combined to create shutdown of many programs vital to hepatocytes. These included decrease in expression of enzymes related to fatty acid metabolism, urea cycle, cell replication, and mitochondrial functions and increase in expression of glycolysis enzymes. There was however increase in expression of genes of plasma proteins. Hepatocyte average volume decreased to 35% of control with proportional decrease in dimensions of the hepatic lobules. Mice died at 15-18 days after hepatectomy with ascites, increased plasma ammonia and very small livers.
Profound dysregulation of oxidative stress pathways in METKO+EGFRi mice was also evident. LncRNA profiling in METKO+EGFRi mice indicates differential regulation of LncRNAs regulating proliferation, transcription and translation. Decreased expression of genes regulating cell proliferation, DNA replication and metabolism in METKO+EGFRi mice was also evident. Key enzymes involved in lipid homeostasis like SCD1, AMPK, ACC, ELOVL3 and mTOR were markedly inhibited in METKO+EGFRi mice resulting in reduced lipogenesis, membrane fluidity and cellular proliferation.
Conclusion: The study shows that MET and EGFR separately control many non-overlapping signaling endpoints, allowing for compensation when only one of the signals is blocked. The combined elimination of the signals however is not tolerated. The results provide critical new information on interactive MET and EGFR signaling and the contribution of their combined absence to regeneration arrest and liver decompensation.

MICROBIOME

M1 Orthologs of an Autoantigen in the Human Microbiome as Cross-reactive Triggers of Systemic Autoimmunity

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Background: The earliest autoantibodies in systemic lupus erythematosus (SLE), a prototypical autoimmune disease, are directed against the RNA binding autoantigen Ro60 followed by epitope spreading to other autoantigens. The origin of the initial triggers of Ro60 autoantibodies are unknown. We identified commensal Ro60 orthologs in human skin, oral, and gut microbiomes and hypothesized that Ro60-ortholog-carrying commensals induce autoimmunity via cross-reactivity with human Ro60 in genetically susceptible individuals.
Methods: V4 16S rDNA samples from SLE and control subjects were sequenced using 2x250bp paired-end reads on the MiSeq platform. Samples were also tested for species-specific enrichment for Ro60 bacteria (*Propionibacterium propionicum, Corynebacterium amycoluatum*, and *Bacteroides thetaiotaomicron)* by real-time PCR. Co-immuniprecipitation was performed using human SLE serum and Ro60 ortholog-containing bacterial lysates. A northern blot of the resulting RNA was probed for bacterial Y RNA. Memory CD4 T cells from SLE patients were sorted into CCR6+ and CCR6- populations. Ro60-specific T cells were cloned using a T cell library assay and stimulated with heat-killed Ro60 bacteria. Ro60$^{-/-}$ mice were crossed to the TLR7.1 C57BL/6 transgenic lupus model and screened for Ro60 antibodies. Germ-free mice were monocolonized with *B. thetaiotaomicron* and tested for anti-Ro60 antibodies by ELISA. Mesenteric lymph node (MLN) and spleen cells were stimulated with bacterial and human Ro60 to assess for proliferation in vitro.
Results: Ro60-producing gut commensals were prevalent in controls and lupus patients. However, when human serum was used to co-immmunoprecipitate Ro60 and its bound Y RNA from the Ro60+ skin commensal *P. propionicum*, only antibodies from human Ro60-positive lupus patients reacted with commensal Ro60. Lack of binding in Ro60-negative patients or healthy controls supported antibody cross-reactivity between human and commensal Ro60. Further, Human Ro60-specific CD4 memory T cell clones positive for the tissue-homing marker CCR6 proliferated in response to *P. propionicum*, demonstrating T cell cross-reactivity with commensal Ro60. Serum anti-Ro60 IgG autoantibodies were persistently induced in Ro60$^{-/-}$ TLR7 tg mice, suggesting a microbial trigger in the absence of host Ro60. Finally, germ-free C57BL/6 mice were monocolonized with *B. thetaiotaomicron*. MLN and splenic lymphocytes from monocolonized mice proliferated in response to bacterial and human Ro60 and sera contained anti-human Ro60 IgG antibodies.
Conclusions: Anti-human Ro60 IgG in Ro60-/- TLR7 tg mice suggests a microbial trigger of these autoantibodies. Monocolonization with Ro60+ bacteria induced cross-reactive anti-human Ro60 responses *in vivo*. Ortholog cross-reactivity is underscored by Ro60-specific T cell clones and sera from lupus patients that reacted with commensal Ro60 in vitro. Our data support a model in which colonization with Ro60 ortholog-carrying skin and gut bacteria sustain chronic autoreactivity in lupus. Quantifying and targeting Ro60+ bacteria in SLE patients may lead to novel biomarkers and treatment approaches. Ortholog cross-reactivity is a novel concept that could contribute to the pathogenesis of human autoimmune diseases more broadly.

M2 Microbiome-mediated Suppression of Inflammation-associated Colon Carcinogenesis by Histamine-producing *Lactobacillus reuteri*

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Colorectal cancer (CRC) is the third most common cancer and leading cause of cancer related mortality. Population-based cohort studies have shown that patients with inflammatory bowel disease (IBD) have an increased lifetime risk of CRC compared to the general population. The role of the intestinal microbiome in CRC development has recently been investigated, and manipulation of the gut microbiome by probiotics was shown to suppress CRC in rodent models. Histidine decarboxylase (HDC) deficiency has been shown to promote inflammation-associated CRC by accumulation of CD11b^{+}Gr-1^{+} immature myeloid cells (IMCs). Here, we address the ability of histamine-producing probiotic lactobacilli to complement histamine deficiency in *Hdc*$^{-/-}$ mice by inter-kingdom complementation. We demonstrate that the gut microbe *hdc*$^{+}$ *Lactobacillus reuteri* ATCC PTA 6475 converts histidine to histamine in the mouse intestine. Evidence includes significantly increased colonic *hdc* mRNA and fecal histamine quantities in *Hdc*$^{-/-}$ mice. In an azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced inflammation-associated CRC model, administration of *L. reuteri* 6475 diminished the numbers and sizes of colon tumors and colonic uptake of [18F]fluorodeoxyglucose (FDG) by positron emission tomography (PET) in *Hdc*$^{-/-}$ mice. Administration of *L. reuteri* 6475 suppressed KC, IL-22, IL-6, TNF, and IL-1α gene expression in colonic mucosa and reduced the amounts of pro-inflammatory, cancer-associated cytokines, KC, IL-22 and

IL-6, in plasma. *L. reuteri* 6475 also decreased the relative numbers of $CD11b^{+}Gr\text{-}1^{+}$ IMCs in the spleen. Collectively, histamine generating *L. reuteri* 6475 suppresses inflammation-associated colon carcinogenesis. The anti-tumorigenic effect of *L. reuteri* 6475 was further compared with its isogenic histidine decarboxylase (*hdcA*)-deficient strain. Oral intake of *hdcA*-deficient *L. reuteri* which is unable to generate histamine did not yield increased fecal histamine quantities in $Hdc^{-/-}$ mice. In the AOM/DSS-induced CRC model, *hdcA*-deficient *L. reuteri* did not suppress colon carcinogenesis in $Hdc^{-/-}$ mice compared with wild type *L. reuteri* 6475. These results indicate that the enzymatic machinery, histidine decarboxylase, must be present in *L. reuteri* 6475 to generate histamine as the bioactive compound *in vivo* for the suppression of colonic tumorigenesis. Our findings link luminal conversion of dietary components by gut microbes and probiotic-mediated suppression of CRC. Microbiome-mediated suppression of carcinogenesis may open new avenues for identification of therapeutic targets and for prevention strategies in oncology, and may provide clues for discovery and development of next generation probiotics.

M3 Method Matters: Extraction Approach Influences Bacterial Community

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Microbiome characterization is now highly relevant in the clinical management of fecal microbial transplant procedures, and as such, there is a continued need for standardization of processes. The Human Microbiome Project compared specimens from different human body sites using a modified Mo Bio Power Soil Kit (Mo Bio Laboratories, Carlsbad, CA) protocol.

Focusing on stool specimens, we evaluated both extraction kit and homogenization of the specimen. Homogenization has been suggested for stool processing as it may provide the opportunity for more accurate sampling compared to scraping a single area of the provided stool. Based on previous findings related to extraction methods and effect of homogenization, we compared the pooled scrapings from three different sites of the stool specimen to that of homogenized specimen (1:2 ratio) using molecular grade water. Stool specimen was homogenized using a Smasher (3 times for 60s at highest setting). We immediately extracted the bacterial DNA from stool scrapings and homogenized stool using three different protocols: Mo Bio Power Soil kit protocol, modified HMP protocol, and Mo Bio Power Fecal kit protocol. DNA concentrations and purity were determined. Amplification and sequencing of the V1V3 region of the 16S rRNA gene was performed using the NEXTflex™ 16S V1V3 Amplicon-Seq Kit 2.0 (Bio Scientific, Austin, TX) with 20 ng of input DNA, and sequences were generated on the Illumina MiSeq platform (Illumina, San Diego, CA). Sequence data was processed through the LotuS pipeline. Briefly, reads were de-multiplexed, and paired ends were stitched. Quality filtering was performed prior to operational taxonomic unit (OTU) clustering utilizing a modified version of the UPARSE algorithm. Taxonomic assignment was performed with RDP as the classifier, HitDB and SILVA databases. OTUs failing to classify as Bacteria at the kingdom level were removed before further analysis.

Most of the OTU's belonged to Bacteriodetes (49.8%) and Firmicutes (41.4%). Statistical analysis of taxonomic and functional profiles (STAMP) was employed for the visualization and statistical analysis of OTUs. Principal component analysis (PCA) was utilized to visualize overall differences between the protocols. Based on the PCA plot, the bacterial community is slightly more diverse in homogenized specimens compared to that of scrapings. The Power Fecal kit designed specifically for fecal materials detects a greater number of bacteria based on OTUs, but for specimen types other than fecal, the Power Soil kit continues to be the most effective. In summary, Mo Bio's Power soil and Power fecal kit were more advantageous than the modified HMP protocol in terms of detecting a more diverse bacterial community, and the protocols for the aforementioned kits are less labor-intensive in regards to hands-on time and total processing time.

M4 Gut Microbiome and Inflammation: A Study of Inflammasome Knockout Mice

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Diabetes is a disease characterized by high glucose levels, increased CRP, pro-inflammatory cytokines, and monocyte activation. Recently, the role of the gut microbiome as a potential contributor to the prevalence of diabetes has surged with the recent advances in microbial DNA sequencing technologies. Microbes, or endogenous molecules produced by bacterial communities, activate inflammatory responses through the pattern recognition receptors. Previous work has shown an increase of TLR2 and TLR4 pattern recognition receptors in a diabetes STZ-induced murine model, as well as TLR2 and TLR4 knockouts resulting in decreased microvascular complications. In addition to pattern recognition receptors, the inflammasome is another major innate signaling pathway. NLRP3 is an intracellular Nod-like receptor of inflammasomes that is also activated by bacterial molecules as pore forming toxins, particulate matter, ATP, and RNA. Hindering inflammasome activation has previously demonstrated to decrease inflammation. To date, no studies have focused on the effect of diabetes-induced inflammation and the intestinal microbiome in NLRP knock-out mice.

Briefly, three groups of mice were used: wild-type (WT), WT with STZ (diabetic) and NLRPKO with STZ (diabetic with knock out of NLRP) to test the effect of the intestinal microbiome on diabetes. Blood glucose, and biomarkers of inflammation (SAA, Il-6,IL-18) in mice was measured, and stool was collected after 24 weeks of diabetes. Microbial DNA was extracted from the stool using the MO BIO PowerSoil extraction kit and amplification and sequencing of the bacterial V1V3 region of the 16S rRNA gene was performed using the NEXTflexT 16S V1V3 Amplicon-Seq Kit 2.0. DNA sequences were generated on the Illumina MiSeq platform with a minimum of 800 and average of 7500 sequences generated per sample. The LotuS OTU processing pipeline was employed to provide denoising and phylogenetic reconstruction. Reads were demultiplexed and paired-ends were stitched. A modified version of UPARSE was used for quality filtering and taxonomic assignment was performed by RDP, HitDB, and SILVA. Any OTU not classifying as Bacteria at the kingdom level were omitted from further analysis.

Principal component analysis (PCA) was used to distinguish the differences between the murine groups and alpha diversity was calculated by the Shannon or Chao index. Alpha diversity showed no significant difference between the control (WT), WT with STZ, and NLRP knockout groups with STZ. PCoA of the 16S OTUs using UniFrac reveals a distinction between the control group and the WT with STZ group, as well as distinction between WT with STZ and the NLRP knockout with STZ. The taxonomic distribution reveals a significant increase in Firmicutes and a decrease in Bacteroidetes in the WT with STZ mouse group in comparison to the controls. In addition, there was a significant increase in the Firmicutes to Bacteroidetes in the WT with STZ that was restored in the NLRP knockout with STZ group.

Results suggest that selective members of the intestinal microbiome are affected in an STZ-induced diabetes murine model. The bacterial communities are also altered by NLRP knock out mice, which further correlates to the decreased inflammation observed in a diabetic milieu.

M5 Distinct Microbiome Neuroimmune Signatures Correlate with Functional Abdominal Pain in Children with Autism Spectrum Disorder

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Background: Emerging data on the gut microbiome in autism spectrum disorders (ASD) suggest that altered host-microbe interactions may

contribute to disease symptoms. Although gut microbial communities in children with ASD are reported to differ from individuals with neurotypical development, it is not known whether these bacteria induce pathogenic neuroimmune signals.

Methods: Because commensal Clostridia interactions with the intestinal mucosa can regulate disease-associated cytokine and serotonergic pathways in animal models, we evaluated whether microbiome-neuroimmune profiles differed in ASD children with functional GI disorders (FGID), as compared to neurotypical (NT) children with and without abdominal pain. Paired rectal biopsy and blood specimens were collected from 35 children (ASD-FGID (n=14), NT-FGID (n=15), and NT (n=6)). Microbial 16S rDNA community signatures, cytokines and serotonergic metabolites were quantified and correlated with gastrointestinal symptoms.

Results: A significant increase in mucosa-associated Clostridiales, specifically *Clostridium lituseburense*, *Lachnoclostridium bolteae*, *Lachnoclostridium hathewayi*, *Clostridium aldenense*, and *Oscillospira plautii* was observed in ASD-FGID; whereas marked decreases in *Dorea formicigenerans* and *Blautia luti*, as well as *Sutterella* species were evident. Stratification by abdominal pain revealed eight organisms in ASD-FGID that correlated significantly with cytokines (IL-6, IL-1, IL-17A, IFN-γ): *Turicibacter sanguinis*, *C. aldenense*, *C. lituseburense*, *O. plautii*, *Clostridium disporicum*, *Clostridium tertium, Tyzzerella* species and *Parasutterella excrementihominis*. Group comparisons revealed that IL-6 and tryptophan release by mucosal biopsies was highest in ASD children with abdominal pain, whereas serotonergic metabolites were generally elevated in children with FGIDs. Furthermore, proinflammatory cytokines correlated significantly with several Clostridiales previously reported to associate with ASD, as did tryptophan and serotonin.

Conclusions: Our findings identify distinctive mucosal microbial signatures in ASD children with FGID that correlate with cytokine and tryptophan homeostasis, known mediators in central and peripheral pain sensitization. Future studies are needed to establish whether these disease-associated Clostridiales species confer early pathogenic signals in children with ASD and FGID.

M6 Microbial-derived Short-chain Fatty Acids Induce Host Interleukin-10 Receptor and Augment Epithelial Barrier Function

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The interactions between the enteric microbiota and distal gut play important roles in regulating human health. Short chain fatty acids (i.e. butyrate) produced by anaerobic microbes represent a major energy source for the host colonic epithelium. A decreased concentration of luminal butyrate has been strongly associated with colonic disease, including inflammatory bowel disease.

Recent work from our lab has revealed that butyrate enhances intestinal trans-epithelial electrical resistance (TEER) and barrier through stabilization of the hypoxia-inducible factor (HIF-1α). Interestingly, the anti-inflammatory interleukin-10 receptor (IL10R), expressed on the apical side of the intestinal epithelium, is also important for barrier formation. These findings have led us to hypothesize that butyrate is augmenting intestinal barrier through the IL10R pathway.

Using human intestinal epithelial cells (IECs), we have discovered that butyrate induces IL10R expression and enhances intestinal barrier formation. Studies in germ free mice revealed decreased IL10R in the colonic epithelium supporting our hypothesis that microbial-derived butyrate is important for IL10R expression. Lentiviral knockdown of the IL10R in IECs led to decreased TEER and barrier formation. Conversely, overexpression of IL10R led to enhanced epithelial resistance and barrier formation. The addition of trichostatin A, a histone deacetylase (HDAC) inhibitor, to IECs increased IL10R expression suggesting that butyrate is mediating the receptor through its role as an HDAC inhibiter. We are currently investigating how butyrate and interleukin-10 receptor over-expression increase intestinal epithelial barrier by examining the expression of cell junction proteins. Our current results indicate that microbial-derived butyrate participates in a diverse set of functions in the gut: regulation of the interleukin-10 cytokine receptor, barrier formation; it could even be a potential therapeutic for mucosal protection.

MUCOSAL PATHOBIOLOGY

MP1 Intestinal Epithelial Expression of CD47 Facilitates Cell Proliferation, Migration, and Mucosal Wound Closure *In Vivo*.

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The cell membrane glycoprotein CD47 is a ubiquitously expressed 'self' antigen that is known to facilitate a wide variety of interactions involving hematopoietic cells depending on the cell type and context. Specifically, CD47 has been reported to regulate the induction of apoptosis, leukocyte transmigration, and non-classical recognition/phagocytosis of cells by macrophages. Despite mounting evidence on CD47 function in leukocytes, comparatively little is known about the function of CD47 expression by non-hematopoietic cells. Previous studies suggest that epithelial expression of CD47 is important for rapid neutrophil transepithelial migration and cell matrix adhesion *in vitro,* yet the function of epithelial expressed CD47 has not been explored *in vivo*.

In this study, we utilized both epithelial-targeted (Villin-Cre CD47$^{f/f}$) and tamoxifen-inducible epithelial-targeted (Villin-Cre-ER^{T2} CD47$^{f/f}$) transgenic mice to assess the function of CD47 in the intestinal epithelium *in vivo*, both under homeostatic conditions and during intestinal inflammation. Gross intestinal mucosal architecture and barrier integrity of Villin-Cre CD47$^{f/f}$ mice was normal under steady state conditions *in vivo*, while acute loss of intestinal epithelial CD47 expression in Villin-Cre-ER^{T2} CD47$^{f/f}$ mice produced no change in mucosal function at one month post-tamoxifen treatment. However, evaluation of crypt epithelial cell proliferation revealed decreased EdU uptake in CD47-deficient epithelial cells, with accompanying alterations in proliferation- and survival-associated signaling. Moreover, both Villin-Cre CD47$^{f/f}$ mice and tamoxifen-treated Villin-Cre-ER^{T2} CD47$^{f/f}$ mice displayed greater susceptibility to an injury induced (DSS) model of chronic colitis. Consistent with our previous *in vitro* findings of delayed neutrophil transmigration upon antibody-mediated blockade of epithelial CD47, fewer epithelium-associated neutrophils were recovered from inflamed colons of Villin-Cre CD47$^{f/f}$ mice *in vivo*. Finally, both epithelial-targeted CD47 deficient mice and total CD47 knockout mice experienced significantly delayed wound healing in an endoscopic biopsy model of mucosal injury, with reduced epithelial cell migration and proliferation in crypts immediately adjacent to wounds.

These findings provide novel insights into the function of CD47 expression by non-hematopoietic cells in the regulation of normal epithelial cell proliferation and turnover, while enabling rapid recruitment of neutrophils under inflammatory conditions. Furthermore, given the current appreciation of CD47 as a non-classical regulator of "don't eat me" signals such that cells deficient in CD47 are cleared by splenic macrophages, our observations that mice harboring epithelial-targeted CD47 deficiency are healthy indicate that additional regulatory mechanisms mediate CD47 function.

NEUROPATHOLOGY

N1 Metallo-Biology of Amyloid-Beta **(Aβ)** Plaque Cores from Alzheimer´s Disease

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Metals play a significant role in neurobiology, particularly in neurodegenerative diseases. Abnormal accumulation of brain metals is a key feature of Alzheimer's disease (AD). Especially, iron, copper and zinc bind to Aβ, these interactions result in aggregation and precipitation into APC. High affinity of Aβ for metal ions is correlated with accumulation of Fe and their eventual aggregation into more stable particulate forms. These events are a possible mechanism of neurons associated to mitigate increased oxidative stress and misbalance of brain metals. Formation of amyloid-β plaque cores (APC) is related to interactions with biometals,

especially Fe, Cu and Zn, but their particular structural associations and roles remain unclear. The advanced or high resolution imaging of metalloproteins has been a great challenge, but recent progress on instrumentation, detector and design has allowed to reveal fine details of complex hybrid samples.
In particular, we established an integrative set of advanced transmission electron microscopy (TEM) techniques, including spherical aberration-corrected scanning transmission electron microscopy (Cs-STEM), nano-beam electron diffraction, electron holography and analytical spectroscopy techniques (EDX and EELS) for the high-resolution imaging and spectroscopy characterization of amyloid plaque cores from AD. Here we demonstrate that Fe in APC is present as iron oxide (Fe3O4) magnetite nanoparticles. Importantly, we show that Fe was accumulated primarily as nanostructured particles within APC, whereas Cu and Zn were distributed through the amyloid fibers. These results were also confirmed by synchrotron based scanning transmission X-ray microscopy. Remarkably, these highly organized crystalline magnetite nanostructures directly bound into fibrillary Aβ showed characteristic superparamagnetic responses with saturated magnetization with circular contours, as observed for the first time by off-axis electron holography of nanometer scale particles. The magnetic contours of magnetite corresponded to the mean inner potential and no lines of magnetic force were observed, which in fact is in agreement with the lower critical size for the superparamagnetic response and consistent with our previous observations from the collective content of APC.
The presence of magnetic nanoparticles associated with AD reveals the importance of metallobiology of human neurons, as a key point for identification of misbalanced processing/transport of metal ions in neurodegenerative diseases.

N2 CX3CR1 and DAP12-TREM1 and -TREM2 Signaling in Multiple Sclerosis versus Alzheimer's Disease: Biosimulations of Proteomics Differences and Effects of a Novel Orally Active CX3CR1 Blocker (AZD8797)

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TYROBP differential gene expression is a causal regulator for immune and microglia response in late-onset Alzheimer's disease (LOAD). TREM2 and CX3CR1, in this response pathway, were minor nodes in the signaling network. Reactome.org includes TYROBP and TREM1 and TREM2 in the DAP12 signaling pathway, and CX3CR1 in the chemokine (fractalkine) and G-protein coupled receptor pathways, associated with G-alpha 'i', 'q', and 'z'. Microglia are considered CNS 'sweepers' and tissue homeostasis 'gatekeepers' in LOAD and multiple sclerosis (MS); implicating IL-34 and Csf1 receptor mechanism for DAP12 activation (cell survival), TREM2 and DAP12 signaling (phagocytosis), CD200 (resting), and both the CX3CR1-dependent ERK signaling (CNS chemotaxis recruitment) and cAMP inhibitory pathways.
Our study utilized Transcriptome-To-Reactome™ (TTR™) technology for neuron-microglia interaction biosimulations to examine differences between LOAD and MS using COPASI software and determining kinetic parameters from NCBI GEO transcriptome sets GSE28146 and GSE38010. The TTR™ also tested an [IC_{50}] (350nM; Cederblad et al., 2016) effect of AZD8797 (CID11956767 - AstraZeneca's developmental non-competitive allosteric CX3CR1 inhibitor to treat MS) to normalize ERK and cAMP biomarkers in both MS and LOAD subjects, *in silico*. Autodock-Vina was used for ligand-protein docking and proteomic heatmap results were generated with Cluster and Treeview. Principal component analysis was performed with R using the FactoMineR library. LOAD as mild, moderate, and severe were clustered with age-matched control, but separated from control, acute and chronic MS, CNS-white-matter, plaques, which were separated from each other.
Heatmaps revealed distinctions among the groups and showed signs of normalization by AZD8797. Fractalkine sequentially binds two sets of residues in the extracellular pocket of CX3CR1, activating G-proteins; AZD8797 binds the secondary residues. AZD8797 increases cAMP levels and lowers ERK markers by approximately 72% and 42%, respectively. Microglial activation pathways are not identically altered in LOAD and MS, but both diseases might be treatable with novel drugs targeting CX3CR1.

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Campen, Matthew	A2
Canady, Lauren C	L8
Cantu, Concepcion C	ID4, ID7
Carano, Richard A	IM3
Castro, Nadia P	C7
Causero, Araldo	IN4
Chang, Yu-Chan	C5, C6
Chen, Ming-Huang	C6
Chen, Xinguo	M1
Chen, Xinpu	M4
Chowdhury, Ananda	C7
Chun-Nan, Yeh	C6
Cifu', Adriana	IN4
Coleman, William B	C3
Colgan, Sean	M6
Consitt, Leslie	A1
Contreras, Deisy	ID1, ID2
Cossu, Carla	C1, L5, L6
Curcio, Francesco	IN4
Dalal, Prarthana	IN2, IN3
Danilenko, Dimitry M	IM3
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DeFrances, Marie C	L16
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DeLeo, Frank R	ID4
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DeMorrow, Sharon	L1, L2, L3, L8
Deveraj, Sridevi	M4
Di Benedetto, Paolo	IN4
Doersch, Karen M	IM4
Domenis, Rossana	IN4
Dostal, David E	L9
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Ehrlich, Laurent	L9
Fabris, Martina	IN4
Faburay, Bonto	ID6
Fan, Jia	C12, ID3
Flores, Anthony R	ID7
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Gangalapudi, Vineela	ID2
Gao, Chunxu	M2
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Hazen, Meredith	IM2
Holers, Michael	IM1
Horstmann, Nicola	ID3
Hötzel, Isidro	IM2, IM3
Hsiao, Michael	C5, C6
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Hu, Ye	ID3
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Jialal, Ishwarlal	M4
Jian, Payandeh	IM2
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Kuhn, Kristi	M6
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Langley, Farris	IM1
Lee, Wyne	IM2
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Li, Yunzhou	C9
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Matsumoto, Marissa L	IM2
Mayorga, Jesus Paez	ID7

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Nasser, Waleed	ID7
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Newell-Rogers, M K	IM4
Nguyen, Tommy	C4
Niazi, Kayvan R	IN4
Norenberg, Jeffrey	A2
Nusrat, Asma	MP1
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Olsen, Randall J	ID4, ID7
Orr, Anne	L12, L16
Owens, Jennifer	L7
Padhye, Masumi	ID1
Pahwa, Roma	M4
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Parker, Joel S	C3
Parkos, Charles	MP1
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Paul Singh Monga, Satdarshan	L14
Peeney, David	C7
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Phung, Thuy L	C4
Pistis, Cinzia	IN4
Plascencia Villa, Germán	N1
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Pradhan-Sundd, Tirthadipa	L14
Puri, Puneet	C9
Qi, Jianjun	C13, C14
Rajapaksa, Kathila S	IM3
Ramaiaha, Arunachalam	ID2
Ramanujan, V. Krishnan	ID1
Randall, Olsen J	ID3
Ranganathan, Saranganajan	L14
Reed, Michelle	MP1
Reif, Karen	IM3
Reif, Karin	IM2
Renfroe, Stephen	M1
Reyes, Arthur	IM3
Richt, Juergen	ID5, ID6
Riva, Ivan De la	ID7
Ruff, William	M1
Runge, Jessica K	M3, M5
Salomon, Art	L11
Salomon, David S	C7
Sameer Padhye, Masumi	ID2
Sanders, Jennifer	L11
Sandhu, Rupninder	C3
Sato, Keisaku	L4
Savidge, Tor	M5
Schaefer, Rachel	M6
Schirtzinger, Erin E	ID5
Schwisow, Kayla	M6
Severs, Erin	M6
Shah, Rajesh R	M2
Shang, Yonglei	IM2
Sharma, Neeraj	IM3
Shelburne, Samuel A	ID3
Shen, Liangfang	C12
Shi, Zhongcheng	M2
Soliman, Karam F	C2, IN1
Solon, Margaret	IM2, IM3
Spissu, Ylenia	C1, L5, L6
Stetler-Stevensson, William G	C7
Sullivan, David	IN2, IN3
Sundd, Prithu	L14
Szymanski, Candice	A1
Taka, Equqr	IN1
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Tao, Wenjing	G1
Tomasi, Maria Lauda	C1, L5, L6
Tomlinson, Stephen	IM1
Trujillo, Jessie D	ID5
Tseng, George C	L16
Vats, Ravi	L14
Veenstra-VanderWeele, Jeremy	M5
Venable, Susan	M2
Venkatachalam, Alamelu	M3, M5
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Vicario, Annalisa	IN4
Vieira, Silvio	M1
Vuopio, Jaana	ID7
Wang, Jianyong	IM2, IM3
Wang, Timothy C	M2
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White, Tori	L9
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Willis, Monte S	CM1, CM2
Wilson, William C	ID5, ID6
Wolin, Sandra	M1
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Xue, Yuhua	L12
Yan, Donghong	IM2
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Zhao, Nianxi	C10, C11
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Zhou, Huiping	C9
Zhou, Lili	L14
Zhou, Tianhao	L4
Zhu, Luchang	ID3, ID7
Zu, Youli	C10, C11, C12, C13, C14, G1

Congratulations to our PISA 2016 Meritorious Award Winners!

ASIP 2017 Rous-Whipple Award

James M. Musser, MD, PhD

ASIP 2017 Robbins Distinguished Educator Award

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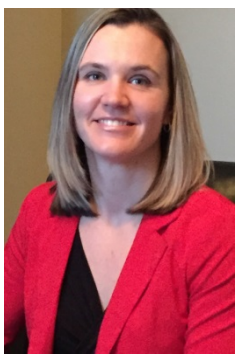

Christi Kolarcik, PhD

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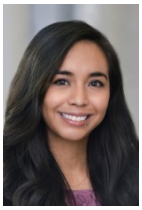
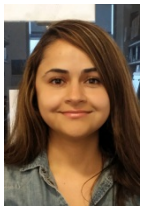


Miriam Balderas, PhD



Deisy Contreras, MS

Adeola Adebayo Michael, PhD

International Trainee Travel Award Recipient

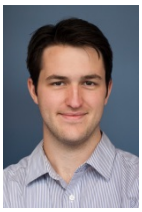
Adenike Christy Adeyemo, PhD

Gall Travel Award for Excellence in Cardiovascular Research Award Recipient

Traci Parry, PhD

Trainee Travel Awards

Trainee Travel Awards Supported by the A.D. Sobel-ASIP Education Fund



Ian Ackers, BS

Ali Alawieh, BS

Cara Bocon, BS

Carla Cossu, BS

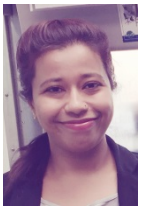
Prarthana Dalal, BA

Carina Anja Dehner, MD

Andrea Floris, BS

Lindsey L. Kennedy, BS

Matthew McMillin, PhD



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April O'Brien, BS

Tirthadipa Pradhan-Sundd, PhD

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Shuanghui Yang, MD

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Cecelia Yates, PhD

American Society for Investigative Pathology
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Biobanking
Daniel Remick, MD
Liliana Guedez, PhD

Breast Cancer
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Ashley G. Rivenbark, PhD

Cell Injury
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Cecelia C. Yates, PhD

Digital and Computational Pathology
Stanley Cohen, MD
John Tomaszewski, MD

Environmental and Toxicologic Pathology
Elizabeth Galbreath, DVM, PhD

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David Williams, MD, PhD
Qin Yan, PhD

Immunohistochemistry and Microscopy
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Nissi Varki, MD

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Satdarshan Paul S. Monga, MD
Kari Nejak-Bowen, MBA, PhD

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Gregory Tsongalis, PhD
Andrei Turtoi, PhD

Neuropathology
Warren G. Tourtellotte, MD, PhD, FCAP
Christi L. Kolarcik, PhD

Pulmonary Pathobiology
Dani Zander, MD

Regenerative Medicine and Stem Cells
Jose Javier Otero, MD, PhD
Bryon Petersen, PhD

Tumor Microenvironment and Metastasis
William Stetler-Stevenson, MD, PhD
Anirban Maitra, MBBS
Piyali Dasgupta, PhD

Vascular and Mucosal Pathobiology
Francis W. Luscinskas, PhD
Asma Nusrat, MD
Dan Milner, MD, MSc, FCAP
Michael Schnoor, PhD, MSc
David P. Sullivan, PhD

Veterinary and Comparative Pathology
Robert Johnson, DVM, PhD
Stacey Fossey, DVM, PhD

(Attending PISA 2016)

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ASIP 2017 Annual Meeting at Experimental Biology

Deciphering the Pathways of Health and Pathology: Informing Biomarker Development, Prevention, Diagnosis, and Treatment of Disease

April 22-26, 2017 - Chicago, IL

Abstract Submission Deadline: November 17, 2016
Travel Award Application Deadline: November 17, 2016

PRELIMINARY PROGRAM

Saturday, April 22, 2017

- **Trainee Welcome Breakfast**
- **Highlights: Graduate Student Research in Pathology,** Chaired by Titus A. Reaves, PhD
- ASIP YOUNG SCIENTIST LEADERSHIP AWARD LECTURE, **A Balancing Act: Role of HNF4α/ß-Catenin Interaction in Hepatobiliary Development and Cholangiocarcinoma Formation,** Chad Walesky, PhD
- **Breast Cancer Workshop: Ductal Carcinoma *In Situ* - Discerning Aggressive Versus Benign Disease Using Molecular Features**
  Chaired by William B. Coleman, PhD
- **Neuropathology of Autonomic Dysfunction**
  Chaired by Warren Tourtellotte, MD, PhD, Jose Otero, MD, PhD
- **XVIIth Annual Workshop on Graduate Education in Pathology: PhD Workforce**
  Chaired by Robin Lorenz, MD, PhD, Dani Zander, MD
- **Course: Pathobiology for Research Scientists: Next-Generation Genomics for the Basic/Translational/Clinical Scientist**
  Chaired by William B. Coleman, PhD, Mark E. Sobel, MD, PhD, Gregory J. Tsongalis, PhD
- **PAMPs and DAMPs: New Roles in Immunopathogenesis**
  Chaired by Asma Nusrat, MD, Andrew Neish, MD
- **ASIP Awards Presentations and ASIP Business Meeting**
  Chaired by George K. Michalopoulos, MD, PhD
- **EB-Wide Welcome Reception**

Sunday, April 23, 2017

- **Committee for Career Development & Diversity Workshop and Breakfast: Developing Your Personal Brand for Career Success**
  Chaired by Luisa DiPietro, DDS, PhD, Magali Saint-Geniez, PhD
- **SCVP Symposium: New Roles for Inflammation in the Heart**
  Chaired by James R. Stone, MD, PhD
- MOLECULAR AND CELLULAR BASIS OF DISEASE: **Liver Pathobiology Symposium: Novel Insights into the Mechanisms of Non-Alcoholic Fatty Liver Disease (NAFLD) and Autoimmune Liver Disease**
  Chaired by Kari Nejak-Bowen, MBA, PhD, Satdarshan Paul S. Monga, MD
- **Biology and Pathobiology of Tissue Barriers**
  Chaired by Asma Nusrat, MD, Andrei Ivanov, PhD
- **XVIIth Annual ASIP/AAA Career Development and Mentoring Program and Lunch: Developing Your Core Message: An Interactive Session on Crafting an Effective Elevator Speech**
  Chaired by Diane Bielenberg, PhD, Chad Walesky, PhD
- **Cell Injury Workshop: Creating a Scar: The Roles of Inflammation in Tissue Remodeling**
  Chaired by Cecelia C. Yates, PhD, Denuja Karunakaran, PhD
- MOLECULAR AND CELLULAR BASIS OF DISEASE: **Presidential Symposium: Liver Pathobiology: Prevention of Hepatic Disease and Regeneration**
  Chaired by George K. Michalopoulos, MD, PhD
- ASIP OUTSTANDING INVESTIGATOR AWARD LECTURE, **Why Autophagy is Important to the Liver**, Xiao-Ming Yin MD, PhD

Monday, April 24, 2017

- **ASIP Town Hall Meeting and Breakfast**
  Chaired by Lisa McFadden
- **HCS Symposium: Imaging Signaling *In Vivo* From Cell Biology to Animal Models**
  Chaired by Margarida Barroso, PhD, Douglas Rosene, PhD
- **Journal of Histochemistry & Cytochemistry Lecture**
  Chaired by Stephen Hewitt, MD, PhD
- COTRAN EARLY CAREER INVESTIGATOR AWARD LECTURE: **Unraveling the Complexity of Drug Resistance in Lung Cancer**, Katerina Politi, PhD
- MOLECULAR AND CELLULAR BASIS OF DISEASE: **Cancer Chemoprevention and Biomarker Development**
  Chaired by William Stetler-Stevenson, MD, PhD, Kevin Gardner, MD, PhD
- **Digital Computational Pathology**
  Chaired by Stanley Cohen, MD, John Tomaszewski, MD
- MOLECULAR AND CELLULAR BASIS OF DISEASE: **ACVP Symposium: Epigenetics and Cancer**
  Chaired by Stacey Fossey, DVM, PhD, Robert Johnson, DVM, PhD
- GOLD-HEADED CANE AWARD LECTURE: **Understanding Vascular Endothelium: A Pilgrim's Progress,** Michael A. Gimbrone Jr., MD
- **Blood Vessel Club™: Endothelial Cell Mechanisms that Regulate Function and Permeability**
  Chaired by Pilar Alcaide, PhD, Jonathon Homeister, MD, PhD

Tuesday, April 25, 2017

- **SIPMeT Symposium: Metabolism and Prevention**
  Chaired by Massimiliano M. Corsi Romanelli MD, PhD
- **Stowell Symposium: Trends in Experimental Pathology: Gone with the Wnt: A Classic Tale of Stem Cells, Cancer and More**
  Chaired by Satdarshan Paul S. Monga, MD, Douglas Stairs, PhD
- **Diseases of the Endoplasmic Reticulum**
  Chaired by Jonathan Lin, MD, PhD, Fumihiko Urano, MD, PhD
- **Scientific Sleuthing of Human Disease for Undergraduate Students and High School Teachers and Students**
  Chaired by Kari Nejak-Bown, MBA, PhD, Martha B. Furie, PhD
- **Society of Toxicologic Pathology Symposium: Challenges in Translation: Cardiovascular Modeling as an Exemplar for *In Vitro* to *In Vivo* Extrapolation**
  Chaired by Elizabeth Galbreath, PhD, DVM
- **Targeting Transcription Regulation in Disease**
  Chaired by Philip Iannaccone, MD, PhD, Qin Yan, PhD, David Williams, MD, PhD
- **Scientific Interest Group Networking Sessions and Poster Discussions**

Guest Societies

- American Society for Matrix Biology
- American College of Veterinary Pathologists
- American Physician Scientists Association
- Association of Pathology Chairs
- The Histochemical Society
- The Society for Cardiovascular Pathology
- Societá Italiana di Patologia e Medicina Traslazionale
- Society of Toxicologic Pathology

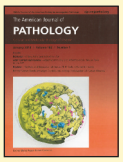
American Society for Investigative Pathology

Investigating the Pathogenesis of Disease

MEMBERSHIP APPLICATION

MEMBER BENEFITS

- ***Scientific Meetings & Courses*** - With reduced registration fees for ASIP members



- ***The American Journal of Pathology (AJP)*** Published monthly in print and online formats. Members receive online access with an option to add a print subscription at a substantially reduced rate.

- ***The Journal of Molecular Diagnostics (JMD)*** Published six times/year in print and online formats. Members receive online access with an option to add a print subscription at a substantially reduced rate.
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- The Society newsletter, ***ASIP Pathways***, features science policy reports on current issues affecting biomedical researchers, as well as news from Committees and members, and other articles of interest.
- ***FASEB Directory of Members*** ASIP member names and contact information included in the online FASEB Directory of Members, including 125,000 scientists and engineers of the 30 represented FASEB member societies
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Please submit your application to:

American Society for Investigative Pathology
Attn: Membership Department
9650 Rockville Pike, Suite E133
Bethesda, MD 20814-3993 (USA)
Phone: (301) 634-7130, Fax: (301) 634-7990
Email: membership@asip.org
web: www.asip.org/apply/

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Job Title

Institution

Department

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Date of Birth *(Month/Day/Year)*

Certifications (Please List)

Degrees:

❒ PhD ❒ DVM
❒ MD ❒ DO
❒ MD/PhD ❒ Other_____

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Ethnicity (Optional Information):

❒ Asian ❒ Native American
❒ Black/African American ❒ Caucasian
❒ Hispanic or Latino ❒ Pacific Islander or Alaska Native
❒ Do not wish to disclose

❒ Other_____

Scientific Interest Groups

Please check all that apply:

❒ Biobanking
❒ Breast Cancer
❒ Cell Injury
❒ Digital and Computational Pathology
❒ Environmental and Toxicologic Pathology
❒ Gene Expression
❒ Immunohistochemistry and Microscopy
❒ Inflammation/Immunopathology
❒ Liver Pathobiology
❒ Molecular Diagnostic Pathology
❒ Neoplasia/Growth Regulation
❒ Neuropathology
❒ Pulmonary Pathobiology
❒ Regenerative Medicine and Stem Cells
❒ Tumor Microenvironment and Metastasis
❒ Vascular and Mucosal Pathobiology
❒ Veterinary and Comparative Pathology

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Applications are reviewed and subject to approval in accordance with the ASIP Bylaws. Your membership benefits will commence on the first day of the month following approval of your application. Your membership will be valid for 12 months from the date of commencement. *Have questions about this application? Email membership@asip.org.*

**OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)*


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