UNIVERSITY *of* ROCHESTER
MEDICAL CENTER

Department of Pathology and Laboratory Medicine
39th Annual Research Day
June 7, 2021
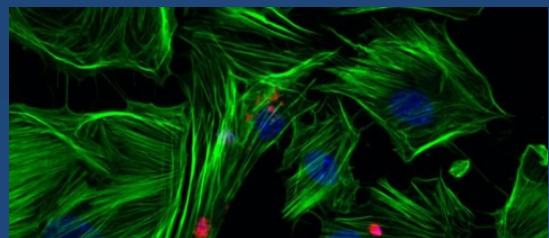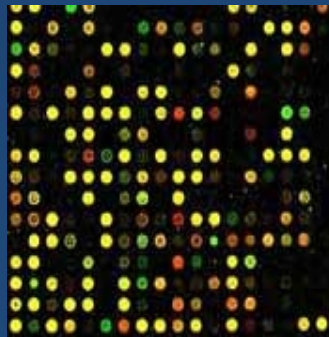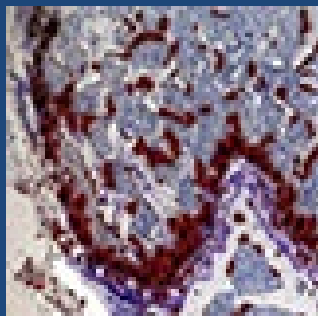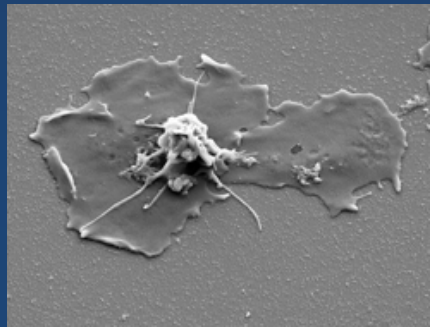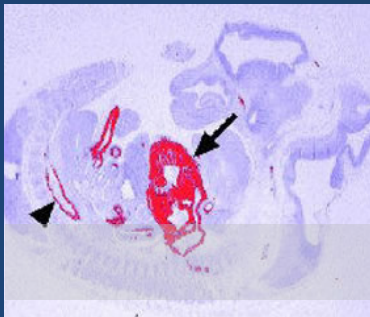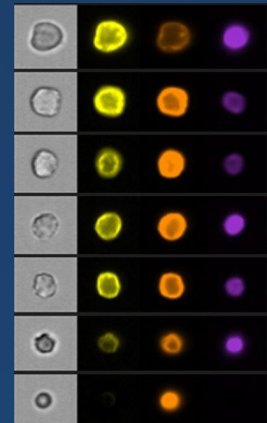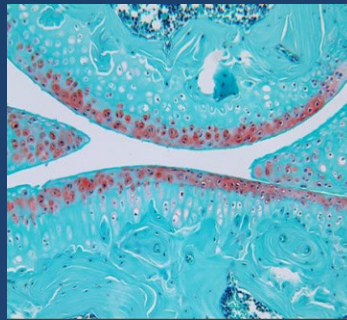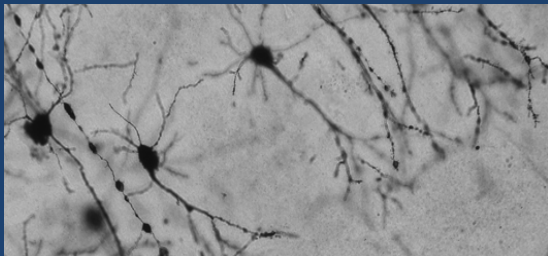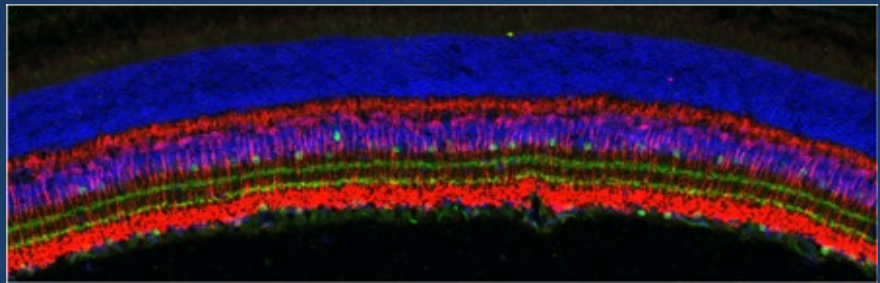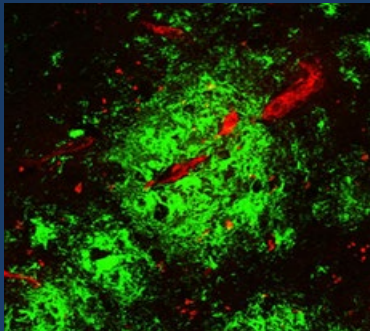
2021 Pathology Research Day

Keynote Lecture

James L. Kirkland, MD, PhD

"Aging, Chronic Diseases, Cellular Senescence, and Senolytics: The Path to Translation."

James L. Kirkland, MD, PhD is the Director of the Robert and Arlene Kogod Center on Aging and the Noaber Foundation Professor of Aging Research at Mayo Clinic in Rochester, MN.

Dr. Kirkland's research focuses on the impact of cellular aging (senescence) on age-related dysfunction and chronic diseases. Senescent cells accumulate with aging and in chronic diseases such as dementia, cancers, diabetes, and arthritis. Dr. Kirkland's team developed the idea that removing senescent cells may extend health span partly based on the observation that mice with mutations that increase lifespan have a lower senescent cell burden than normal mice, and that short-lived mice have more of these cells. To test this idea, Kirkland and his team, in collaboration with others at Mayo Clinic, eliminated senescent cells from genetically modified mice. They found that this process enhanced health span, at least in the context of an accelerated aging-like disease, giving proof of principle for the notion that clearing senescent cells with a drug in non-genetically-modified individuals might be beneficial.

Kirkland's team is using its findings to develop senolytic interventions that delay the onset of aging and alleviate or partially reverse age-related chronic diseases such as diabetes, atherosclerosis, cancers and arthritis among others. "We predict many more senolytic drugs will appear at an accelerating pace over the next few years and that these drugs will be improved to more effectively target senescent cells. These three drugs, if effective in clinical trials, could be transformative," said Kirkland of three new senolytic agents his team uncovered in March 2017.

Pathology Research Day
Monday, June 7, 2021

Schedule of Events

All presentations and poster sessions are virtual presentations. Links provided within schedule

Zoom link: https://urmc.zoom.us/j/97812515923 PLATFORM PRESENTATIONS

8:15 AM   Welcome ~ Dr. Bruce Smoller, Professor and Chair, Pathology and Laboratory Medicine
Dr. Helene McMurray, Diector of PhD Program,
Dr. Linda Schiffhauer, Director, Residency Program
Dr. Jennifer Findeis-Hosey, Vice Chair of Education, Pathology and Laboratory Medicine

8:30 AM   Oral Presentations

Irene Chen, MD (PGY2): *SWI/SNF Chromatin Remodeling Complex in Pancreatic Ductal Adenocarcinoma: Clinicopathologic and Immunohistochemical Study of 353 Cases*

Xiaoqin Liu, MD, PhD (PGY1): *Assessment of Prognostic Factors of Solitary Fibrous Tumors Arising from the Gastrointestinal Tract andLiver: A Clinicopathologic Study of 34 Cases*

Felicia Gilels, MS: *The role of JAG1-mediated Notch signaling in the maintenance and function of cochlear sensory cells.* **Advisor and Department:** Amy E. Kiernan, PhD, Dept. of Ophthalmology

Alexandra Danakas, MD (Cytopathology Fellow): *Comparison of Diagnostic Utility of Delta-Like Ligand 3 to Insulinoma-Associated protein 1 (INSM1) in Neuroendocrine Neoplasms of Lung*

9:20 AM   Juried Poster Session ~ Musculoskeletal Diseases ~ Helene McMurray
Zoom Link: https://urmc.zoom.us/j/95151858315

10:05 AM   *Break*

10:15 AM   Oral Presentations: https://urmc.zoom.us/j/97812515923 PLATFORM PRESENTATIONS

Chauncey Syposs, DO (PGY2): *Differential Expression of DLL3 in Merkel Cell Carcinoma Primary Tumors and Metastases*

Anthony Cardillo, MD (PGY2) *In-Silico Testing: Accurately Predicting Results of the Direct Antiglobulin Test using Commonly Available Lab Data*

Sara Blick-Nitko, MS: *The Multifactorial Roles of Platelets in Uncomplicated Malaria Infection* **Advisor and Department:** Craig Morrell, DVM, PhD, Dept. of Medicine, Aab CVRI

Hani Katerji, MD (Breast/GYN Fellow): *Risk stratification for Management of Breast Cancer Patients using Genomic Testing in Needle Core Biopsies:Learning During the COVID-19 Pandemic and Future Implications*

11:05 AM   Juried Poster Session ~ Cancer ~ Majed Refaai, MD
              Zoomlink: https://urmc.zoom.us/j/95151858315

11:50 AM   Lunch Break

12:30 PM   Keynote Address: Introductions:
              Zoomlink: https://urmc.zoom.us/j/95151858315

              James L. Kirkland, MD, PhD

              "Aging, Chronic Diseases, Cellular Senescence, and Senolytics: The Path to Translation."

              Director of the Robert and Arlene Kogod Center on Aging and the Noaber Foundation
              Professor of Aging Research Mayo Clinic, Rochester, MN

1:30 PM   Juried Poster Session ~ Hematology and Immunology ~ Linda Schiffhauer, MD
              Zoomlink: https://urmc.zoom.us/j/95151858315

2:15 PM   Break

2:25 PM   Oral Presentations
              Zoomlink: https://urmc.zoom.us/j/97812515923_PLATFORM PRESENTATIONS

              Bennett Wilson, DO (PGY4): *Negative Urine Cytology and the Rate of Diagnostic
              Agreement between Cytotechnologist and Cytopathologist: Why Not Let
              Cytotechnologists Sign-Out Negative Urines?*

              Cynthia Reyes Barron, MD (Dermatopathology Fellow): *Evaluation of Melanocyte Loss in
              Mycosis Fungoides Using SOX10 Immunohistochemistry*

              Jiatong Liu, MS: *Callus Senescent Cells Inhibits Aging Fracture Via TGFb1*
              Advisor and Department: Lianping Xing, PhD, Dept. of Pathology and Laboratory Medicine

3:05 PM   Juried Poster Session ~ Cell Biology and Genetics ~ Jennifer Findeis-hosey, MD
              Zoomlink: https://urmc.zoom.us/j/95151858315

3:55 PM   Closing Remarks:
              Dr. Bruce Smoller, MD, Chair, Department of Pathology and Laboratory Medicine
              Zoomlink: https://urmc.zoom.us/j/97812515923_PLATFORM PRESENTATIONS

Pathology Research Day
Monday, June 7, 2021
Juried Poster Session ~ 9:30am
Musculoskeletal Diseases

Zoomlink: https://urmc.zoom.us/j/95151858315_POSTER SESSIONS

- ❖ 1 - Jessica Ackerman, MS, PGY 2017
  The periostin niche as a target for modulating myofibroblast differention to promote regenerative tendon healing

- ❖ 2 - M. Nick James, MS, PGY 2018
  Dissecting the role of DNA double-strand breaks in osteoarthritis pathogenesis

- ❖ 3 - Kiana Chen, PGY 2019
  Elucidating Testosterone Effects on Myeloid Cell Populations in Inflammatory Arthritis

- ❖ 4 - Katherine Escalera-Rivera, PGY 2019
  Cartilage-synovium crosstalk in osteoarthritis: role of NR4A1 signaling

- ❖ 5 - H. Mark Kenney, MS, MSTP, PGY 2019
  Joint-draining popliteal lymphatic vessels exhibit lymphatic muscle cell dysfunction in TNF-Tg mice with inflammatory arthritis

- ❖ 6 - Meghan O'Neil, PGY 2019
  Characterization of RAGE as a therapeutic target for myofibroblast inhibition during the remodeling phase of tendon healing

- ❖ 7 - Yue Peng, PGY 2019
  Investigating Lymphatic Muscle Cell Turnover during TNF-Induced Inflammatory Arthritis

- ❖ 8 - Chen Yu, PGY 2019
  Transcriptional Regulation of Cyclophilin-D During Adipogenic Differentiation

- ❖ 9 - Cynthia Reyes Barron, MD, ~ Dermatopathology Fellow
  Comparison of the Genetic Mutations in Sporadic and BRCA1 Carrier Breast Cancer Through Targeted Next Generation Sequencing

Callus Senescent Cells Inhibits Aging Fracture Via TGFβ1

Jiatong Liu[1], Hengwei Zhang[1], Brendan F. Boyce[1,2], Lianping Xing[1,2]

[1]Department of Pathology and Laboratory Medicine; [2]Center for Musculoskeletal Research, University of Rochester, Medical Center, Rochester, NY, USA

Bone fractures in the elderly can lead to serious health issues and also is a financial burden for the healthcare system. Currently there are no FDA proved drugs for enhancing fracture healing. Fracture healing involves angiogenesis and osteogenesis, both of which require sufficient number of mesenchymal stem/progenitor cells (MPCs) that is reduced during aging. A recent study by Joseph et al., reported decreased proliferation and increased senescent phenotype in bone marrow MPCs isolated from aged mice following fracture. However, the contribution of cell senescence to aged fracture remains to be investigated. Previously, we reported that the senolytic drugs, dasantinb + quercetin, enhanced fracture healing in aged, but not in young mice, and conditioned medium (CM) collected from aged callus inhibited the growth of callus-derived MPCs (CaMPCs). Here, we aimed to identify critical senescent associated secretory phenotype (SASP) factors in fracture callus of aged mice with the hypothesis that callus senescent cells (SCs) produce excessive transforming growth factor beta 1 (TGFβ1), which inhibits the proliferation of CaMPCs, and this can be prevented by TGFβ neutralization. We used young 3-m-old and aged 18-m-old C57BL/6J mice and tibial fracture model. Compared to CM collected from young (3-m) mouse callus, CM from aged mouse callus expressed high levels of active form of TGFβ1 proteins and inhibited the growth of CaMPCs, which was prevented by a TGFβ1 neutralizing antibody. High TGFβ1 expression were confirmed in SCs isolated from callus as well as protein extracts of total callus tissues of aged mice. Treatment of fractured aged mice with the TGFβ1 neutralizing antibody promoted the fracture healing indicated by histology and biomechanics, accompanied by increased callus MPC numbers and cell proliferation. Our study identifies TGFβ1 as an important SASP factor that is produced by callus SCs in aged mice and inhibits MPC proliferation. Local blockade of TGFβ1 signaling during the early phase of fracture healing may represent a new therapeutic strategy for fracture healing in the elderly.

Jessica Ackerman, MS

Program Year: 2017

Advisor and Department: Alayna Loiselle, PhD, Dept. of Orthopaedics, Ctr for Musculoskeletal Research

The periostin niche as a target for modulating myofibroblast differention to promote regenerative tendon healing

Jessica Ackerman[1,2], Alayna Loiselle,[2,].

[1]Department of Pathology and Laboratory Medicine, University of Rochester, Medical Center; [2]Department of Orthopaedics, Center for Musculoskeletal Research,

Tendon injuries are a major clinical problem, with poor patient outcomes due to tendon's fibrotic healing process. A main driver of fibrosis is thought to be persistence of matrix-producing myofibroblasts. While myofibroblasts are required for structural support and wound contraction following tissue injury, aberrant accumulation can initiate fibrosis via pathological matrix production. Periostin is a matricellular protein that has been linked to the progression of fibrosis in a number of other tissues, and is utilized in the heart to label matrix-producing myofibroblasts. We hypothesized that postn+ cells likewise represent activated myofibroblasts in the context of acute tendon injury, and aimed to better understand the relationship between periostin and α-SMA+ myofibroblasts to define therapeutic targets to attenuate fibrotic tendon healing.

To determine the potential of Postn+ cells to differentiate into α-SMA+ myofibroblasts, Postn-Cre[ER]; Ai9 mice underwent surgical repair of the the FDL and were put on a diet of tamoxifen chow throughout healing to continuously label Postn-lin cells with tdTomato fluorescence. Following harvest at D28, Postn-lin cells are observed abundantly at the injury site, but we noted essentially no colocalization with α-SMA expression, contrary to our hypothesis that these cells become α-SMA+ myofibroblasts in the context of tendon injury. Instead, they may provide an important supportive function, possibly through secretion of periostin into the scar tissue matrix.

To better understand the role of secreted periostin following tendon injury, we conducted co-immunofluorescence for both secreted protein and α-SMA in C57BL6/J mice from D3 to D28 after surgical repair. Interestingly, at D7 and 14, α-SMA+ myofibroblasts are found within a dense network of secreted periostin, suggesting that it may act as a niche for myofibroblast differentiation and/or function.

Next we investigated if a periostin matrix could induce tenocyte differentiation *in vitro*. Recombinant postn was added to the collagen I coating, and tenocytes grown on this matrix significantly differentiated to a-SMA+ myofibroblasts after 24h compared to collagen alone (8% increase, p=0.002), reinforcing this idea of a periostin niche for MF differentiation.

Collectively, these data suggest that both Postn+ cells and the secreted protein have distinct supporting roles during fibrotic healing, and that targeting the periostin myofibroblast niche may present a novel therapeutic strategy to reduce scarring during tendon healing.
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M. Nick James, MS

Program Year: 2018

Advisor and Department: Jennifer Jonason, PhD, Dept. of Orthopaedics

Dissecting the role of DNA double-strand breaks in osteoarthritis pathogenesis

M. Nick James[1], Sarah E. Catheline[1], and Jennifer H. Jonason[1,2].

[1]Department of Pathology and Laboratory Medicine, URMC; [2]Department of Orthopaedics and Rehabilitation, URMC

Osteoarthritis (OA), the most common and most expensive form of arthritis, is characterized by progressive loss of articular cartilage, however, the exact molecular mechanisms responsible for OA onset are still unclear. Aging is known to be among the most important risk factors associated with OA development. However, while aging is thought to be a consequence of accumulated DNA damage, the relationship between DNA damage and OA is not clear. Our laboratory has found that aged articular chondrocytes have increased inflammatory NF-κB activation and that enhanced IKKβ/NF-κB activation in chondrocytes accelerates spontaneous OA development in relatively young mice. DNA double-strand breaks (DSBs), a particularly toxic form of DNA damage, are also known to activate IKKβ which, in turn, promotes inflammatory NF-κB signaling. Thus, we hypothesize that accumulation of DNA damage in aging leads to chronic proinflammatory signaling that ultimately results in degeneration of articular cartilage and OA onset. To investigate the inflammatory role of DNA damage in OA development, we treated ATDC5 chondroprogenitors with the topoisomerase inhibitor etoposide which induces DNA DSBs. We demonstrated that etoposide effectively produces accumulation of DNA DSBs in ATDC5 chondroprogenitors and increases NF-κB activation by western blot. Etoposide treatment also increased in vitro expression of NF-κB and IRF gene targets including proinflammatory cytokines, catabolic matrix enzymes, and interferon-stimulated genes. We are currently in the process of developing a genetic mouse model which allows inducible, cartilage-specific induction of DNA DSBs through the intron-encoded endonuclease IPpoI. As proof of concept, we transfected ATDC5 cells with an expression vector encoding NLS-I-PpoI which resulted in increased proinflammatory and hypertrophic chondrocyte gene expression. These results suggest that DNA damage-induced inflammation may play a role in OA development. In the future, we plan to use in vivo, cartilage-specific, IPpoI-induced DNA damage to study the effects of DNA damage on OA development. Understanding the impact of DNA DSBs on OA pathogenesis could inform development of novel OA therapeutic strategies that interrupt signaling pathways which mediate inflammation in OA.

Poster Category: Musculoskeletal Diseases -- 3

Kiana Chen

Program Year: 2019

Advisor and Department: Homaira Rahimi, MD, Dept. of Pediatrics, Pediatric Rheumatology

Elucidating Testosterone Effects on Myeloid Cell Populations in Inflammatory Arthritis

Chen, K[1], Bell, RD[1,2], Schwarz, EM[1,2,3], Rahimi, H[3,4]

[1]Department of Pathology; [2]Department of Orthopedics; [3]Center for Musculoskeletal Research,
[4]Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Rheumatoid arthritis (RA) is characterized by chronic joint inflammation and is female predominant. The TNF-transgenic (TNF-Tg) mouse model of RA develops inflammatory arthritis. Previously, we showed selective sexual dimorphism as females die significantly earlier than males, and as joint disease progression differs between same age sexes. However, the mechanisms behind this discrepancy has yet to be determined. Clinical studies suggest sex hormone differences is a possible explanation, with androgens providing a protective effect against disease progression. We hypothesized that androgens' anti-inflammatory effects on myeloid cells in the joint mediate inflammatory arthritis progression.
Orchiectomies were performed on three 4-week old male TNF-Tg mice. At 3-months old, testosterone and TNF-α levels were compared between orchiectomized and age-matched sham mice, and knee joints were H&E and TRAP stained for histological scoring. Bone marrow-derived macrophages (BMDMs), plated from an 8-month old TNF-Tg-Tg male and 3-month old TNF-Tg-Tg female, underwent treatment with 0.1µM, 1µM, and 10µM of testosterone propionate and LPS stimulation. Cell culture supernatant was assayed in an ELISA for murine TNF-α.

Orchiectomized TNF-Tg mice had significantly lower testosterone levels than sham controls, and no difference in TNF-α levels. Orchiectomized mice joints had significantly higher histology scores than sham controls for synovial inflammatory infiltrate, pannus invasion, and TRAP+ area. Although statistical significance could not be determined, the 8-month old male TNF-Tg mouse's BMDMs exhibited a notable decrease in TNF-α concentration in the 0.1µM testosterone-treated stimulated plate .

These preliminary results suggest that endogenous testosterone removal in TNF-Tg mice does not affect the TNF-α production responsible for their disease phenotype, and that there is a TNF-α independent earlier onset of joint disease in orchiectomized mice. The *in vitro* results suggest that testosterone treatment may have a potential effect on the pro-inflammatory activity of stimulated TNF-Tg macrophages, which will be further determined with expanded BMDM experiments.

Poster Category: Musculoskeletal Diseases -- 4

Katherine Escalera-Rivera

Program Year: 2019

Advisors and Department: Jennifer Anolik, MD, Dept. of Medicine, Div. of Allergy, Immunology and Rheumatology; Jennifer Jonason, PhD, Dept. of Orthopaedics, CMSR

Cartilage-synovium crosstalk in osteoarthritis: role of NR4A1 signaling

Katherine Escalera-Rivera[1,2], Jennifer Anolik[3], Jennifer Jonason[4].

[1]Department of Pathology; [2]Center for Musculoskeletal Research; [3]Department of Medicine Division of Allergy, Immunology and Rheumatology; [4]Department of Orthopaedics, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Osteoarthritis (OA) is a leading cause of disability in aging adults. OA is characterized by the loss of articular cartilage but affects all tissues within the joint including the synovium. Proinflammatory cytokines contribute to OA pathogenesis by mediating cartilage extracellular matrix catabolism. The transcription factor NF-κB is an effector of proinflammatory signaling and its activity is increased in OA chondrocytes. Moreover, it is a target of regulatory networks that control inflammation. NR4A1 is an orphan nuclear receptor that participates in such a network. The role of NR4A1 in preventing inflammatory signaling in the joint, however, is not fully understood. Preliminary data from our lab shows that NR4A1 protein expression is altered in cartilage and synovium from human subjects. When compared to a healthy control, NR4A1 expression decreases in the cartilage surface and increases in synovium from OA patients. Furthermore, we also observed these changes in protein expression in knee joints from 27-month-old C57BL6/J mice, which develop an osteoarthritic phenotype. By optimizing a protocol to disaggregate mouse synovial tissue and performing flow cytometry on the isolated cells, we found an increase in the total amount of immune cells within the knee joint synovium when compared to young C57BL6/J mice. We hypothesize that absence of NR4A1 in the chondrocytes during OA progression leads to uncontrolled secretion of proinflammatory cytokines and immune cell infiltration to the synovium. Preliminary data from our lab shows that *in vitro* knockdown of *Nr4a1* in chondrocytes leads to upregulation of *Ccl20* and *Il-6*, both of which are NF-κB target genes and are involved in the recruitment of immune cells. On-going experiments will determine if global deletion of *Nr4a1* in mice affects OA progression. In addition, we will develop i*n vitro* co-culture systems to define the effects of *Nr4a1* expression in chondrocytes on neighboring synovial cells and select immune cell populations.

Poster Category: Musculoskeletal Diseases -- 5

H. Mark Kenney, MS, MSTP

Program Year: 2019

Advisor and Department: Edward M. Schwarz, PhD, Dept. of Orthopaedics, CMSR

Joint-draining popliteal lymphatic vessels exhibit lymphatic muscle cell dysfunction in TNF-Tg mice with inflammatory arthritis

H. Mark Kenney,[1,2] Yue Peng,[2] Lianping Xing,[1,2] Christopher T. Ritchlin,[2,3] Edward M. Schwarz[1,2,3,4]

[1]Department of Pathology & Laboratory Medicine; [2]Center for Musculoskeletal Research [3]Department of Medicine, Division of Allergy, Immunology, Rheumatology; [4]Department of Orthopaedics; University of Rochester Medical Center

Previous studies demonstrated that tumor necrosis factor transgenic (TNF-Tg) mice with inflammatory arthritis exhibit altered ultrastructure of lymphatic muscle cells (LMCs) and eventual loss of popliteal lymphatic vessel (PLV) contractions associated with severe joint disease. Remarkably, anti-TNF therapy in flaring TNF-Tg mice recovers these PLV contractions concomitant with amelioration of arthritis (1). As this regenerative process is critical for resumption of joint function, knowledge regarding the mechanisms of LMC failure and recovery in inflammatory arthritis is needed. Thus, we tested the hypothesis that alpha smooth muscle actin (αSMA)[+] PLV-LMC coverage is reduced in severe arthritis, and anti-TNF therapy reinstates LMC integrity through turnover of bromodeoxyuridine (BrdU)[+] PLV-LMCs associated with return of joint homeostasis.

To assess LMC coverage and turnover, we administered BrdU (i.p. 0.1mg/g/day) to 8-month-old WT and TNF-Tg (placebo or anti-TNF therapy; i.p. 10mg/kg/week) mice for 6 consecutive weeks followed by PLV harvest with multicolor whole mount immunofluorescent microscopy for αSMA and BrdU, as previously described (2). During treatment, mice were monitored for talus bone volumes by micro-CT at 3-week intervals as an established outcome of joint disease.

Our preliminary findings suggest αSMA[+] PLV-LMC coverage was reduced in TNF-Tg placebo treated (60.45 ± 22.84%) compared to WT mice (86.15 ± 10.04%), while anti-TNF therapy demonstrated a recovery trend (75.38 ± 12.56%; p=0.0589 vs. TNF-Tg placebo). Surprisingly, increased αSMA coverage appeared to be unrelated to LMC turnover, as all groups had <2% BrdU[+]/αSMA^{+} cells. Increased αSMA[+] PLV-LMC coverage with anti-TNF therapy was associated with recovery of talus bone volumes.

Overall, chronic inflammation leads to reduced αSMA[+] PLV-LMC coverage, and anti-TNF therapy appears to recover αSMA[+] PLV-LMCs associated with joint regeneration. Given the insignificant contribution of LMC turnover in lymphatic recovery, future work will validate initial success in single-cell RNA-sequencing of LMCs using recently described LMC lineage tracing models (2) to characterize LMC pathology and novel treatment targets for inflammatory arthritis.

1. Bouta et al. *A&R*. 2017. PMC5449211
2. Kenney et al. *Sci Rep*. 2020. PMC7581810

Meghan O'Neil

Program Year: 2019

Advisor and Department: Alayna Loiselle, PhD, Dept. of Orthopaedics

Characterization of RAGE as a therapeutic target for myofibroblast inhibition during the remodeling phase of tendon healing

Meghan M. O'Neil[1,2], Alayna E. Loiselle, PhD[1,2,3]

[1]Department of Pathology and Laboratory Medicine; [2]Center for Musculoskeletal Research, URMC; Department of Orthopaedics & Rehabilitation, URMC; [3]Department of Biomedical Engineering, URMC

Acute tendon injuries heal via a scar-mediated process that is often complicated by excessive scar tissue formation causing functionally impaired tendons. After inflammation occurs, myofibroblasts (MFs) are responsible for synthesizing and contracting matrix to promote tissue continuity and cell migration during the fibroblastic phase of healing. Continued myofibroblast activity into the remodeling phase causes the rate of matrix formation to be greater than the rate at which matrix is degraded, which forms excessive scar tissue. **We hypothesize that myofibroblasts need to be active during the fibroblastic phase to deposit and contract matrix to promote proper healing, but then need to be inhibited during the remodeling phase to block excessive scar formation.** One mechanism of persistent MF activity is from continued expression of pro-survival factors from NF-kB signaling. However, NF-kB has limited therapeutic targets due to its ubiquitous presence. To elucidate a therapeutic target that will inhibit myofibroblast activity during the remodeling phase, we will focus upstream of NF-kB on the receptor RAGE. S100a4 acts as a secreted signaling molecule that binds to RAGE and has shown to influence NF-kB activity in many tissues. We first need to show that S100a4-RAGE acts upstream of NF-kB in tendon. *In vitro* C57BL/6J tenocytes will be treated with S100a4 recombinant protein and RAGE antagonist peptide (RAP) to show that inhibition of S100a4-RAGE inhibits NF-kB activity. Then we need to show that inhibition of S100a4-RAGE will lead to inhibition of pro-survival factors from NF-kB signaling. We will cross RAGE[F/F] mouse to a aSMA-CreER mouse to induce knock-out of RAGE in myofibroblasts. NF-kB activity will be assessed by quantification of p65 and phosphorylated p65 and pro-survival factors will be assessed using western blots. Taken together, we will be able to characterize the receptor RAGE as target for inhibiting myofibroblast activity during the remodeling phase of tendon healing.

Yue Peng

Program Year: 2020

Advisor and Department: Helene McMurray, PhD, (*ad hoc*), Dept. of Pathology and Laboratory Med.

Investigating Lymphatic Muscle Cell Turnover During TNF-Induced Inflammatory Arthritis

Yue Peng,[1,2] H. Mark Kenney,[1,2] Edward M. Schwarz[1,2,]

[1]Department of Pathology & Laboratory Medicine; [2]Department of Orthopaedics; University of Rochester Medical Center, Center for Musculoskeletal Research;

Rheumatoid arthritis (RA) is an immune-mediated inflammatory disorder characterized by progressive synovitis. Previous studies with the tumor necrosis factor-transgenic (TNF-Tg) mouse model of RA showed that progression of knee inflammation is associated with reduced joint-draining popliteal lymphatic vessel (PLV) contractility and lymphatic clearance. These defective PLVs also have decreased alpha smooth muscle actin (αSMA)$^+$ lymphatic muscle cell (LMC) coverage, which might explain the ultrastructural tissue damage in TNF-Tg PLVs. Interestingly, anti-TNF therapy was shown to recover PLV contractions and ameliorate joint disease. Based on this, we hypothesized that LMC turnover is increased during chronic arthritis, and loss of αSMA$^+$ LMC coverage and PLV dysfunction occurs due to peri-PLV progenitor cell depletion from TNF exposure. To test this, we first validated bromodeoxyuridine (BrdU) labeling methods of LMC turnover in neonatal PLVs. We also aimed to develop a live isograft model to assess adoptive transfer of genetically labeled peri-lymphatic LMC progenitors into WT host mice.

To assess LMC turnover, neonatal mice (1-month-old) with known LMC incorporation were treated with BrdU (0.1 mg/g, intraperitoneal) for 7 or 14 consecutive days, and quantification of BrdU$^+$/αSMA$^+$ LMCs relative to total PLV area was compared directly to aged mice (10-month-old) that never received BrdU by whole mount immunofluorescent microscopy. For adoptive transfer studies, we generated Platelet Derived Growth Factor Receptor Beta (PDGFRβ)-CreER x Ai9^{tdTomato} (fluorescent donor) and PDGFRβ-CreER x DTA (ablated LMC recipient) mice treated with tamoxifen (0.1 mg/g, intraperitoneal) for 5 consecutive days as adoptive transfer models. PLVs in these mice were evaluated by in vivo near-infrared (NIR) imaging of indocyanine green (ICG) (recipients) and ex vivo fluorescent microscopy (donors & recipients).

Our results showed that numbers of BrdU$^+$/αSMA$^+$ LMCs increase as expected from 7 (1198.17 ± 525.79 cells/mm^2 PLV) to 14 (2745.28 ± 697.55 cells/mm^2 PLV) days of treatment in neonatal mice, while BrdU$^+$ LMCs were absent in aged negative control PLVs. In addition, the PDGFRβ-CreER x DTA PLVs exhibited loss of contractions and reduced αSMA coverage, and PDGFRβ-CreER x Ai9 PLVs showed ubiquitous tdTomato$^+$/αSMA$^+$ colocalization as potential adoptive transfer models.

In conclusion, we find that in vivo BrdU labeling detects de novo LMC incorporation into PLVs of neonatal mice, which may be used to estimate LMC turnover rate in TNF-Tg mice. Additionally, PDGFRβ-CreER x DTA mice exhibit effective LMC ablation and impaired lymphatic contraction (recipient), while PDGFRβ-CreER x Ai9 demonstrate fluorescent LMCs (donors), which together suggests these mice will be valuable LMC adoptive transfer models. Future studies based on this work will direct a path forward to characterize LMC pathology and novel treatment targets for inflammatory arthritis.

Poster Category: Musculoskeletal Diseases

Chen Yu

Program Year: 2019

Advisor and Department: Helene McMurray, PhD, Department of Pathology and Laboratory Medicine

Transcriptional Regulation of Cyclophilin-D During Adipogenic Differentiation

Chen Yu[1], Owen Charles Smith[2], Rubens Sautchuk[2], Roman A. Eliseev[1,2,3].

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Age-related bone loss has been associated with decreased osteoblast-mediated bone formation, increased bone resorption and accumulation of bone marrow fat. With aging, differentiation potential of bone marrow stromal (a.k.a. mesenchymal stem) cells (BMSCs) is shifted towards adipogenic and away from osteogenic lineage. Our previous data has shown that activation of mitochondria is important during osteogenesis of BMSCs. A byproduct of such an activation is increased ROS, leading to oxidative stress and pathological opening of the mitochondrial permeability transition pore (MPTP) resulting in oxidative phosphorylation (OxPhos) uncoupling, inflammation and even cell death. It is, therefore, beneficial for osteo-induced BMSCs to deactivate this pore. Cyclophilin-D (CypD) is a mitochondrial matrix protein that facilitates opening of the MPTP. We observed that CypD is downregulated during osteogenesis leading to lower MPTP activity and thus protecting mitochondria from ROS. However, the role of mitochondria and CypD during adipogenesis of BMSCs is unclear. Here, we are trying to characterize the potential role of CypD during adipogenesis. Our preliminary data shows that CypD is upregulated during adipogenesis of mouse BMSCs and remains unchanged in human BMSCs. During adipogenesis, BMSCs do not activate mitochondrial respiration but increase glycolysis. Moreover, CypD overexpression in mouse BMSCs enhances adipogenic differentiation. Thus, there appears to be a reciprocity between CypD/MPTP and adipogenic signaling. To study how CypD is regulated during adipogenesis, we performed luciferase promoter reporter assay. Our data shows that CCAAT/enhancer-binding protein alpha (Cebpa) induces transcription of CypD luciferase reporter in mouse BMSCs. And interestingly, p65, one of the NF-kB subunits, shows synergistic effect with Cebpa inducing CypD luciferase reporter expression. For future experiment, we would like to further study metabolic reprogramming during adipogenesis by metabolic tracing; evaluate the effect of manipulation of CypD during the process; and perform ChIP-qPCR to confirm transcription factor binding activity.

Cynthia Reyes Barron, MD

Fellow ~ Dermatopathology

Comparison of the Genetic Mutations in Sporadic and BRCA1 Carrier Breast Cancer Through Targeted Next Generation Sequencing

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Introduction

Women who carry a germline mutation in BRCA1 (breast cancer type 1 susceptibility protein) have an increased risk of developing breast cancer in their lifetimes. Carriers tend to develop breast cancer at a younger age and their tumors are generally aggressive. Although BRCA1 breast cancers have been researched, many questions remain. The purpose of this study was to analyze the mutational landscape of tumors from BRCA1 carriers and compare them to tumors of sporadic breast cancer.

Methods

Pathology reports were reviewed to identify cases of breast cancer (BC) and the ER/PR/HER2 status was noted as well as the Ki67 proliferation index and patient age. Next generation sequencing (NGS) was performed on 72 BC tumors; 26 from BRCA1 carriers and 46 sporadic BC tumors. Two targeted panels were used for sequencing, the Oncomine Comprehensive Assay by Thermo Fisher Scientific on an Ion Torrent S5 XL sequencer and the AmpliSeq for Illumina Cancer HotSpot Panel. Mutations in a total of 44 cancer associated genes common to both panels were analyzed including TP53, RB1, PTEN, NRAS, ERBB2, PIK3CA, and BRAF. Missense and nonsense single nucleotide variants with a variant allele frequency >3% and coverage >150 reads were considered as well as insertions/deletions. Pathogenicity was determined using ClinVar and Varsome. Mutations were classified as benign, likely benign, variants of uncertain significance, likely pathogenic, and pathogenic.

Results

Pathogenic/likely pathogenic variants were identified in 15 genes. TP53 was the most commonly mutated gene with pathogenic/likely pathogenic variants in 13 (50%) BRCA1 carriers' tumors and 18 (39%) sporadic tumors. Having a TP53 mutation did not increase the likelihood of having other pathogenic mutations in either BRCA1 carriers or sporadic tumors; 74% of all tumors with p53 mutations had no additional pathogenic mutations overall. PIK3CA pathogenic/likely pathogenic mutations were significantly more common in sporadic tumors (26%) than BRCA1 carrier tumors (8%). PIK3CA mutation did not increase the likelihood of other gene mutations and did not correlate with TP53 mutations. BRCA1 pathogenic mutations were identified in four cases with no germline testing and likely represent somatic mutations. No

pathogenic mutations were found in 12 (46%) of the BRCA1 carriers' tumors and 10 (22%) sporadic tumors. There were four BRCA1 carriers with bilateral BCs; two pairs had the same mutations, one pair had different TP53 pathogenic mutations and another pair had different PIK3CA pathogenic mutations. A total of twenty-four cases were triple negative, 13 sporadic and 11 BRCA1 carriers (see table below). As expected, BRCA1 carriers were diagnosed with BC at a younger age with 21 (81%) cases diagnosed at age < 60 years while 22 (48%) of sporadic cases were in this age group.

Conclusion

TP53 is the most commonly mutated gene in BCs, even though more common in BRCA1 carriers' tumors; however, they are not required for tumor development as seen in the 57% of cases in our study with normal TP53 (50% of BRCA1 carriers). No specific additional pathogenic mutation was commonly seen in BRCA1 tumors with TP53 mutations. PIK3CA was frequently mutated in sporadic cases and not in BRCA1 carriers. BRCA1 tumors were less likely to have other pathogenic mutations than sporadic tumors in this study of 44 genes. Additional studies will help further elucidate the molecular basis of BRCA1 tumor carcinogenesis.

Genes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Cases
AKT1																																																																								ER+/HER2+
ATM																																																																						ER+/HER2-
BRAF																																																																						ER-/HER2+
CDKN2A																																																																					ER-/PR-/HER2-
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Ki67	25	ND	ND	10	60	5	20	ND	15	40	40	15	ND	ND	20	40	30	ND	25	5	35	70	30	50	80	50	70	80	30	ND	ND	ND	60	40	20	ND	30	ND	20	80	70	15	10	ND	ND	ND	ND	ND	ND	60	70	30	25	5	ND	ND	3	15	60	90	60	90	90	95	90	ND	ND	ND	ND	ND	
Age	56	54	24	67	65	80	71	52	39	40	63	53	65	46	68	66	69	34	35	55	47	21	42	74	63	56	69	64	39	74	69	74	85	58	73	74	38	78	44	49	48	52	66	68	92	82	56	35	35	53	67	59	43	43	79	79	35	49	51	51	70	51	33	43	38	64	51	42	54	46	37	57	age ≥60

Pathology Research Day
Monday, June 7, 2021
Juried Poster Session ~ 11:00 am
Cancer

Zoomlink: https://urmc.zoom.us/j/95151858315 POSTER SESSIONS

❖ 10 – Emily K. Whitt ~ PhD 2019
Effects of SUV420H2 on Breast Cancer Invasion

❖ 11 – Celia Soto ~ PhD 2019
Lactate from Leukemic Cells Drives Macrophage Polarization and Hematopoietic Dysfunction

❖ 12 – Lan Wang ~ PhD 2020
The long non-coding RNA Neat1 confers anti-cancer activity in p53-mediated tumor suppression

❖ 13 – Alexandra M. Danakas, DO ~ Fellow
Expression of Delta-Like Ligand 3 in Thoracic Neuroendocrine Neoplasms: New insights into a Novel Diagnostic and Therapeutic Target

❖ 14 – Cynthia Reyes Barron, MD ~ Fellow, Dermatopathology
Acantholytic Squamous Cell Carcinoma Arising from Lichen Sclerosus: a Rare Case Affecting Vulvar Skin

❖ 15 – Irene Y Chen, MD ~ PGY-2
SWI/SNF Chromatin Remodeling Complex in Pancreatic Ductal Adenocarcinoma: Clinicopathologic and Immunohistochemical Study of 353 Cases

❖ 16 – Xiaoqin (Lucy) Liu, MD ~ PGY-2
Assessment of Prognostic Factors of Solitary Fibrous Tumors Arising from the Gastrointestinal Tract and Liver: A Clinicopathologic Study of 34 Cases

❖ 17 – Chauncey Syposs, DO ~ PGY-2
Differential Expression of DLL3 in Merkel Cell Carcinoma Primary Tumors and Metastases

❖ 18 – Anthony B. Cardillo, MD ~ PGY-2
Fractal Compression: An Old Technique for the New Challenge of Whole Slide Image Storage

❖ 19 – Bennett Wilson, DO ~ PGY4
Negative Urine Cytology and the Rate of Diagnostic Agreement between Cytotechnologist and Cytopathologist: Why Not Let Cytotechnologists Sign-Out Negative Urines?

Poster Category: Cancer -- 10

Emily K. Whitt

Program Year: 2019

Advisor and Department: Paula M. Vertino, PhD, Dept. of Biomedical Genetics, Wilmot Cancer Institute

Effects of SUV420H2 on Breast Cancer Invasion

Emily K. Whitt[1], Paula M. Vertino[2,3,4]

[1]Department of Pathology and Laboratory Medicine, University of Rochester Medical Center
[2]Department of Biomedical Genetics, University of Rochester Medical Center; [3]Wilmot Cancer Institute, University of Rochester Medical Center

Breast cancer metastasis is the leading cause of breast cancer mortality, but little is understood about the mechanisms underlying the progression from primary tumor to metastasis. In the early stages of metastasis, cells from the primary tumor invade the surrounding tissue before breaking away and seeding distant metastases. It has been shown that in some cases tumor cells do not invade and metastasize as single cells, but as clusters of cells through a process known as collective invasion. These clusters use heterotypic cell signaling to invade as a group, with phenotypically distinct leader cells at the tip of the pack, followed by chains of cells termed followers. SUV420H2 is a histone methyltransferase that catalyzes histone H4K20 trimethylation which is associated with transcriptional repression. Loss of H4K20me3 is frequently seen in cancer. Recently, we found that suppression of SUV420H2 in luminal breast cells promotes the acquisition of features typical of migratory cells. To test the role of SUV420H2 and H4K20me3 in collective invasion, we used the drug A196, which depletes cells of H4K20me3, to treat spheroids made of luminal or basal breast cell lines. Cells were seeded for spheroids in media containing A196 for 3 days, then transferred to a matrix and imaged for 2 days to observe invasion. Treatment with A196 promoted increased invasion in both luminal and basal breast cancer spheroids. This suggests that depletion of H4K20me3 may allow cells to adopt a more plastic phenotype which promotes increased collective invasion, perhaps conferring an increased ability to metastasize.

Poster Category: Cancer -- 11

Celia Soto

Program Year: 2019

Advisor and Department: Benjamin Frisch, PhD, Dept. of Patho

Lactate from Leukemic Cells Drives Macrophage Polarization and Hematopoietic Dysfunction

Authors: Celia Soto[1], Maggie Lesch[1,2], Joshua C. Munger[4,5], Benjamin J. Frisch[1,2,3,6]

[1]Department of Pathology; [2]Wilmot Cancer Center; [3]Center for Musculoskeletal Research; [4]Department of Biochemistry and Biophysics; [5]Department of Microbiology and Immunology; [6]Department of Biomedical Engineering

The bone marrow microenvironment provides signals that are vital to hematopoietic stem cell self-renewal and differentiation, both of which are dysregulated in leukemia. This loss of normal hematopoiesis leads to pancytopenia, often fatal in acute myeloid leukemia (AML). Current literature reports that, in solid tumors, the microenvironment polarizes macrophages to a tumor-associated macrophage (TAM) supporting cancer cell survival; yet, little is known about how this occurs in hematological malignancies. We have demonstrated through flow cytometric analysis that bone marrow macrophages in leukemic mice express higher levels of the M2 marker CD206, which has previously been reported as overexpressed in TAMs. Furthermore, cancer cells in solid tumors have been shown to switch their metabolic state to preferentially perform aerobic glycolysis (Warburg effect), producing lactate, which has been tied to macrophage polarization. Similarly, leukemic cells are dependent on aerobic glycolysis, resulting in increased lactate production. In vitro treatment of primary bone marrow macrophages with lactate recapitulated the increased CD206 expression, suggesting lactate in the bone marrow microenvironment as a mechanism of polarization. We and others have previously reported that osteoblastic dysfunction is a key feature of the leukemic bone marrow. In vitro exposure of primary bone marrow mesenchymal stem cells (MSCs) to lactate decreased their proliferation, and decreased their capability to differentiate into mature osteoblastic cells, as measured by CFU-OB assays. This research also aims to profile the metabolic landscape of leukemic marrow in mouse and human AML patient bone marrow plasma samples through LC/MS-based metabolomics. We hypothesize that the increased lactate levels in the bone marrow polarize macrophages to a leukemia-supportive phenotype, and alter other cell types, contributing to disease progression.

The long non-coding RNA *Neat1* confers anti-cancer activity in p53-mediated tumor suppression

Lan Wang[1,2] Stephano S. Mello[2], Zamira G. Soares[2]

[1]Dept of Pathology and Laboratory Medicine, [2]Department of Biomedical Genetics

The p53 gene plays a fundamental role in maintaining genome stability and is a critical tumor suppressor that is found to be mutated many cancers. p53 as a transcription factor induces myriad downstream targets. While most studies focus on protein-coding genes, the non-coding RNAs involved in the p53 anti-tumor network remain elusive. Our lab's previous study has identified the long non-coding RNA *Neat1* as a novel p53 target gene that could play a role in tumor suppression. Our study showed that *Neat1* suppresses transformation in various oncogene-expressing cell lines and its deficiency promotes pancreatic intraepithelial neoplasia (PanIN) lesion in KRras^{G12D} mice, indicating the critical role of *Neat1* in suppressing pancreatic cancer initiation. RNA-seq analysis further showed that *Neat1* deficiency triggers global gene expression changes, suggesting a potential mechanism by which *Neat1* confers tumor suppression activity.

Neat1's mechanism of action in tumor suppression is still unclear, but recent evidence suggests that *Neat1* could play a role in the maintenance of chromatin organization. Notably, the pancreatic lesions observed in *Neat1*^{-/-} mice is strikingly similar to the phenotype of SWItch/Sucrose Non-Fermentable complex (SWI/SNF) deficient mice. SWI/SNF is a chromatin remodeling complex that is important for chromatin structure maintenance and gene expression regulation. Further preliminary data produced by our lab suggests that *Neat1* loss leads to alterations in chromatin accessibility, while also impacting the distribution of SWI/SNF in the chromatin. We therefore hypothesize that *Neat1* directly interacts with chromatin by associating with the SWI/SNF complex and changing epigenomic program to suppress transformation.

To test our hypothesis, we were to utilize the electrophoretic Mobility-Shift Assay (EMSA) to evaluate RNA-protein interaction in vitro. We decided to check for *Neat1*'s interaction with Brg1, a core protein of the SWI/SNF complex. By observing the shift of *Neat1* on gel during electrophoresis caused by changes in mobility upon protein interaction, we would be able to learn if *Neat1* can potentially associate with Brg1 to remodel the chromatin structure. The establishment of EMSA for detecting *Neat1*-Brg1 interaction would allow us to better understand the mechanism by which *Neat1* exerts anti-cancer effect and provide new insights into p53-mediated tumor suppression.

Poster Category: Cancer --13

Alexandra M. Danakas, DO,

Fellow - Cytopathology

Expression of Delta-Like Ligand 3 in Thoracic Neuroendocrine Neoplasms: New insights into a Novel Diagnostic and Therapeutic Target

Alexandra M. Danakas, DO, Moises J. Velez, MD, Chauncey Robert Syposs, DO, Donna K. Russel, Sachica C. Cheris, MD, Tanupriya Agrawal, MBBS, PhD

Department of Pathology & Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Background:
Small cell lung cancer (SCLC) represents approximately 15% of all primary lung cancers. Despite the aggressive nature of advanced SCLC, limited second-line therapeutic options beyond checkpoint inhibitors exist.
Delta-like ligand 3 (DLL3) is an inhibitory Notch ligand which promotes neuroendocrine (NE) tumorigenesis, with recent studies showing that DLL3 is highly expressed in SCLC. The DLL3 expression profile has facilitated the development of new treatment options that use DLL3 to specifically target SCLC cells. There are few studies investigating the role of DLL3 in SCLC, while the literature on other NE tumors is very limited. We aim to provide new insights to the role of DLL3 in identifying NE differentiation and its usefulness as a targeted therapy for NE tumors.

Design:
We identified 72 cases of primary lung tumors diagnosed at our institution from 2016-2020 including both biopsy and resection specimens. These included 33 carcinoid tumors (26 typical and 7 atypical), 12 large cell neuroendocrine carcinomas (LCNEC), 18 SCLC, 2 combined small cell carcinoma and LCNEC, 3 adenocarcinomas and 4 squamous cell carcinomas (SCC). Tissue sections were stained with VENTANA DLL3 (SP347) assay using the recommended staining conditions and tissue controls. DLL3 expression in tumor cells were classified as high expression (≥50%), low (1-49%), or negative (<1%) for expression (Figure 1).

Results:
Positive staining, including high or low expression of DLL3, was found in 58 of the primary lung neoplasms tested, all with NE differentiation. DLL3 expression was present in 73% of typical carcinoid tumors, 71% of atypical carcinoid tumors, all LCNEC and SCLC. No expression was identified in the adenocarcinoma or SCC cases (Table 1). The sensitivity of DLL3 expression is 88%, with a specificity of 100%.

Conclusion:
Our study shows DLL3 expression is comparable to other frequently used NE markers. It shows high expression in SCLC and LCNEC, with a variable expression in majority of carcinoid tumors. DLL3 can be potentially used as a diagnostic marker and a potential therapeutic target for patients with neuroendocrine neoplasms, especially in those with DLL3 high expression, which is observed in all SCLC and LCNEC patients.

Figure 1 legend: Typical carcinoid tumor (H&E, A) with no DLL3 expression (B); Typical carcinoid tumor (H&E, C) with low DLL3 expression (D); Typical carcinoid tumor (H&E, E) with high DLL3 expression (F); Adenocarcinoma (H&E, G) with no DLL3 expression (H). All photomicrographs taken at 400X.

Table 1: DLL3 expression in lung primary neoplasms

Tumor Type	DLL3 Negative n (%)	DLL3 Low Expression n (%)	DLL3 High Expression n (%)
Typical Carcinoid (n=26)	7 (27)	6 (23)	13 (50)
Atypical Carcinoid (n=7)	2 (29)	1 (14)	4 (57)
Large Cell Carcinoma (n=12)	0 (0)	0 (0)	12 (100)
Small Cell Carcinoma (n=18)	0 (0)	4 (22)	14 (78)
Combined Large and Small cell carcinoma (n=2)	0 (0)	0 (0)	2 (100)
Adenocarcinoma (n=3)	3 (100)	0 (0)	0 (0)
Squamous Cell Carcinoma (n=4)	4 (100)	0 (0)	0 (0)

Poster Category: Cancer -- 14

Cynthia Reyes Barron, MD

Fellow ~ Dermatopathology

Acantholytic Squamous Cell Carcinoma Arising from Lichen Sclerosus: a Rare Case Affecting Vulvar Skin

Cynthia Reyes Barron, MD; Sharlin Varghese, MBBS; Tamera Paczos, MD; Bruce R. Smoller, MD

Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

We present the case of an 82-year-old female with acantholytic squamous cell carcinoma affecting vulvar skin. The patient had a history of perineal lichen sclerosus for five years prior to presentation. She was referred to a dermatologist for intractable severe pain associated with the lesions. Biopsiesshowed an infiltrative squamous cell carcinoma with histology consistent with the acantholytic subtype. Acantholytic squamous cell c arcinoma is a rare histologic variant characterized by dyscohesive keratinocytes with pseudoglandular formation and dyskeratosis. It is associated with sun-damaged skin and most commonly occurs in the head and neck of elderly men. Few cases have been reported at non-dermal sites and non-sun exposed dermis. The patient underwent a radical vulvectomy and bilateral inguinal node dissection. The 1.6 cm tumor was diffusely acantholytic and pseudoglands were present (figure A 40x, B 200x). The tumor cells were diffusely positive for p63 immunohistochemical stain (figure C). As expected at this site, there was no solar elastosis identified histologically. However, high grade squamous intraepithelial neoplasia (VIN III) and chronic lichen sclerosus were apparent (figure D 100x). This case represents a rare histologic subtype of squamous cell carcinoma in an unusual site associated with lichen sclerosus instead of solar elastosis.
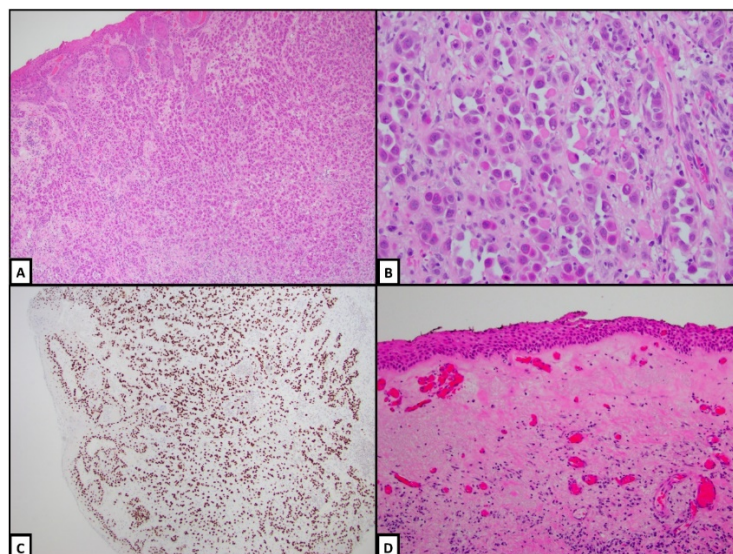
Poster Category: Cancer --15

Irene Y Chen, MD,

Resident: PGY-2

SWI/SNF Chromatin Remodeling Complex in Pancreatic Ductal Adenocarcinoma: Clinicopathologic and Immunohistochemical Study of 353 Cases

Irene Y. Chen, **MD[1],** Mark G. Ettel, MD[1], Phoenix Bell, MD[1], Aaron R. Huber, DO[1], Jennifer J. Findeis-Hosey, MD[1], Wenjia Wang, MD, PhD[1], Aram Hezel, MD[2], Richard F. Dunne, MD[2], Michael G. Drage, MD[1], Diana Agostini-Vulaj, DO[1]

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Background
The Switch/sucrose non-fermentable (SWI/SNF) complex is a multimeric protein involved in chromatin assembly and repair of DNA damage. Mutations of the SWI/SNF complex are observed in approximately one-third of the pancreatic ductal adenocarcinomas (PDACs). Herein, we evaluated the expression of four SWI/SNF complex proteins (ARID1A, SMARCA4, SMARCA2, and INI1) to determine whether SWI/SNF loss is associated with any clinicopathologic features or patient survival in PDAC.

Design
A tissue microarray containing 374 PDACs was stained immunohistochemically using antibodies against ARID1A, SMARCA4, SMARCA2, and INI1. Loss of expression was defined by the complete absence of nuclear staining in tumor nuclei with retained expression in non-neoplastic cells. Clinical and histologic parameters evaluated included patient age, sex, tumor size, margin status, histologic grade, TNM stage, (neo) adjuvant therapy, lymphovascular invasion, perineural invasion, and overall and progression-free survival. Statistical analyses were performed using T-test, Fisher Exact, Kruskal-Wallis, and log-rank tests (p-value <0.05 considered statistically significant).

Results
13 (3.7%) of 353 evaluable PDACs showed deficient SWI/SNF complex expression, which included 11 (3.1%) with ARID1A loss, 1 (0.3%) with SMARCA4 loss, and 1 (0.3%) with SMARCA2 loss. All cases were INI1 proficient. SWI/SNF deficiency was more associated with later onset (median 72 years (range: 58-83)) compared to the SWI/SNF complex proficient PDACs (median 65 years (range 29-88) (p=0.014)). Similar to other cancers, SWI/SNF deficiency was associated with higher histologic grade (**Fig. 1 and 2**) (p=0.030). No other significant clinicopathologic differences were noted between SWI/SNF deficient and SWI/SNF proficient PDACs (**Table**), and no significant differences were seen with respect to overall survival and progression-free survival between SWI/SNF deficient and proficient PDACs (p=0.447 and p=0.439).

Conclusion
In this cohort, SWI/SNF deficiency was seen in 3.7% of PDAC, the vast majority of which were deficient in ARID1A. SWI/SNF deficiency is associated with older age and higher histologic grade, but did not reveal a significant association with prognosis in this aggressive cancer.
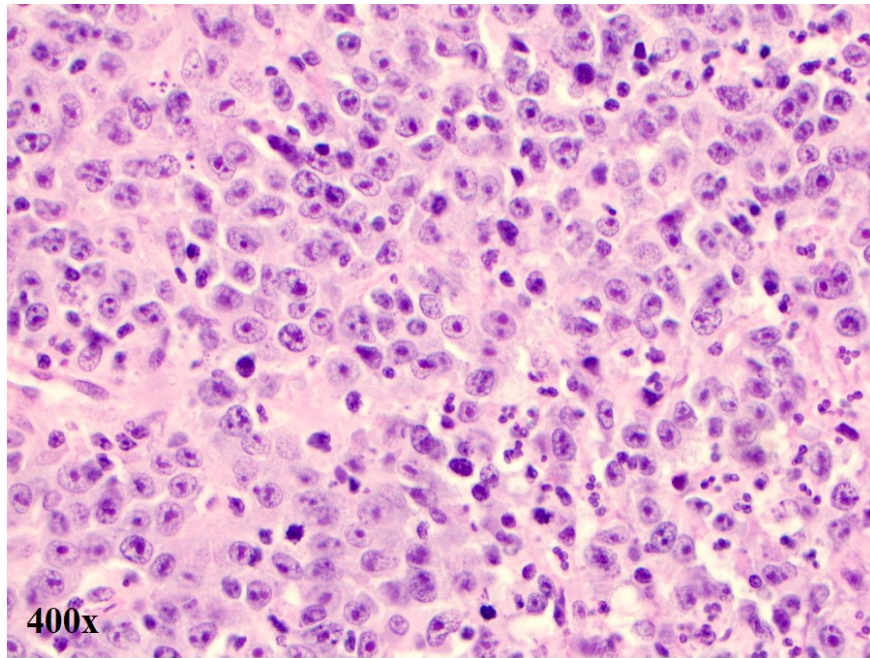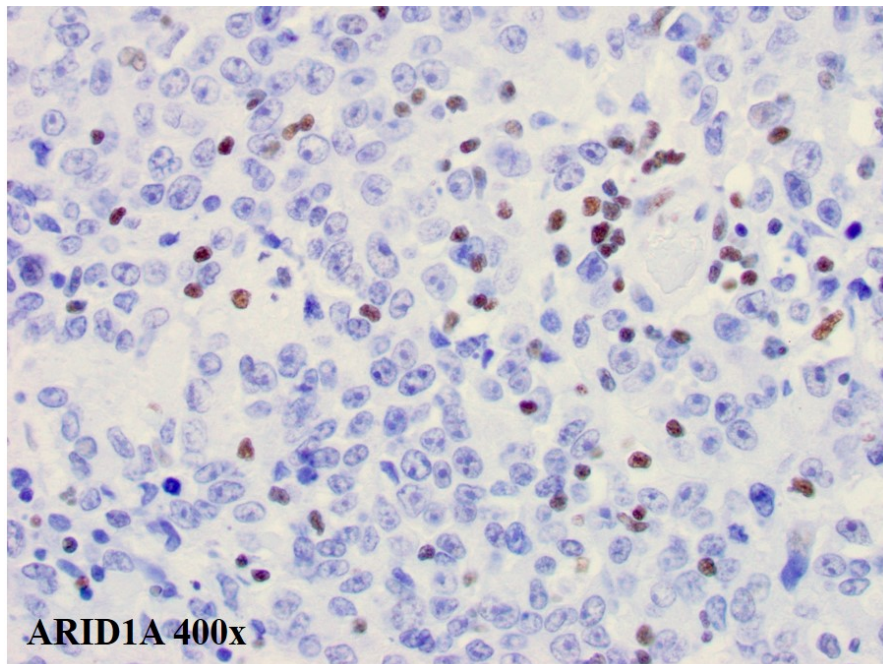
Figure 1

400x

Figure 2

ARID1A 400x

Table

	SWI/SNF Deficient	SWI/SNF Proficient	p-value
Total patients	13	340	
Age, *median (range)*	72 (58-83)	65 (29-88)	0.014
Sex, *n (%)*			0.414
Male	5 (38.5)	172 (50.6)	
Female	8 61.5)	168 (49.4)	
Tumor gross size (cm), *mean ± sd*	3.8 ± 1.2	3.5 ± 1.7	0.361
Margin status			0.779
R0	7 (53.8)	160 (47.1)	
R1	6 (46.2)	180 (52.9)	
Histologic grade			0.030
1	1 (7.7)	33 (9.8)	
2	3 (23.1)	186 (55.0)	
3	9 (69.2)	117 (34.6)	
4	0 (0)	2 (0.6)	
pT stage, *n (%)*			0.928
1a	0 (0)	0 (0)	
1b	0 (0)	1 (0.3)	
1c	1 (7.7)	23 (6.8)	
2	8 (61.5)	216 (63.9)	
3	4 (30.8)	98 (29.0)	
4	0 (0)	0 (0)	
pN stage, *n (%)*			0.766
0	3 (23.1)	99 (29.1)	
1	8 (61.5)	146 (42.9)	
2	2 (15.4)	95 (27.9)	
pM stage, *n (%)*			0.440
0 or X	12 (92.3)	323 (95.8)	
1	1 (7.7)	14 (4.2)	
LVI, *n (%)*	10 (76.9)	203 (60.2)	0.262
PNI, *n (%)*	13 (100)	306 (90.8)	0.618
Treated, *n (%)*			0.085
Neoadjuvant only	2 (22.2)	18 (6.0)	
Adjuvant only	4 (44.4)	220 (73.8)	
Neoadjuvant and adjuvant	0 (0)	12 (4.0)	
No treatment	3 (33.3)	48 (16.1)	

Xiaoqin (Lucy) Liu, MD

PGY-1

Assessment of Prognostic Factors of Solitary Fibrous Tumors Arising from the Gastrointestinal Tract and Liver: A Clinicopathologic Study of 34 Cases

Xiaoqin Liu,MD; Michael-John Beltejar, PhD; Xiaoyan Liao, BMed, PhD

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Background: Solitary fibrous tumors (SFTs) are mesenchymal neoplasms that predominately arise from the pleura. It is usually indolent but can have malignant potential. SFTs primarily involve the gastrointestinal tract and liver (SFTGIL) are extremely rare, and the clinicopathological features remain largely unclear.

Design: A total of 34 SFTGIL, 9 diagnosed at our institution and 25 reported in literature between the years of 2012 and 2020, were included in this study cohort. Three published risk models designed specifically for SFTs [mDemicco, Salas, Pasquali] were evaluated. Statistical analysis was performed by SPSS23. The overall survival (OS) was estimated using the Kaplan–Meier method.

Result: As a group, SFTGILs occurred most commonly in males (n = 25, 73.5%), with a median age of 54 years (range 17–80 y) at initial diagnosis. Twenty-five (73.5%) tumors were of gastrointestinal (GI) origin and 9 (26.5%) were of liver origin. The mean tumor size was 14.6 (range: 1.5-36) cm. Microscopically, the tumor cells were generally bland and spindled, organized in a "patternless" growth pattern, admixed with variable amounts of collagen and typical staghorn blood vessels. Immunohistochemically, STAT6, CD34, and Bcl-2 were positive in 10/10 (100%), 32/34 (94%), and 27/29 (93%) tested cases. Thirty-two (94%) patients were managed with resection, while 2 patients had chemotherapy only. Upon follow-up (median: 65 months), 7 of 34 (20.6%) patients developed tumor recurrences or distant metastasis (3 to lungs, 1 to live), of which 3 (8.8%) patients died of disease progression. Comparisons between tumor origins (GI vs. Liver) showed significant sex differences, in that females were predominant (F: 67%) in hepatic SFT while males were predominant (F: 12%) in GI tract SFT (P<0.01). By univariate analysis, female sex, age (>60y), and tumor necrosis were significant prognostic factors associated with adverse outcome (P<0.05). Hepatic SFTs had worse survival than of GI tract SFTs (p<0.01). Among the three risk models, both Salas and mDemicco, but not Pasquali risk scores, were associated with OS (P=0.025 for Salas, 0.043 for mDemicco).

Conclusion: Our study showed that hepatic SFTs had distinct clinical features and worse survival than GI tract SFTs. The mDemicco and Salas risk models were reliable risk models for identifying patients with SFTGIL at high risk of recurrence or metastasis, which is important to guide clinical follow-up and management.

Poster Category: Cancer --17

Chauncey Syposs, DO

PGY – 2

Differential Expression of DLL3 in Merkel Cell Carcinoma Primary Tumors and Metastases

Chauncey Syposs, **DO;** Anthony Cardillo, MD; Tatsianna Pukhalskaya, MD; Bruce Smoller, MD; Tanupriya Agrawal, MBBS, PhD

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Merkel cell carcinoma is a rare cutaneous neuroendocrine carcinoma associated with high rates of recurrence, metastasis, and poor overall survival. Delta-like Protein 3 (DLL3) is an inhibitory Notch ligand that is currently being developed as a predictive biomarker and potential therapeutic target for neuroendocrine carcinomas. While DLL3 is actively being studied in Small Cell Neuroendocrine carcinomas of the lung, only a single peer-reviewed publication on DLL3 expression in Merkel cell carcinoma currently exists in the English literature. We sought to explore whether DLL3 expression has differential expression in primary cutaneous tumors and lymph node or distant metastases by grading DLL3 expression (no expression, <50% of tumor cells expressing DLL3, >50% of cells expressing DLL3) and staining intensity (low expression versus high expression) in a 18 skin primaries without associated metastatic disease, 10 skin primaries with associated lymph node metastasis, 15 lymph nodes positive for Metastatic --Merkel cell carcinoma, and 8 distant metastatic sites (brain, bone marrow, prostate gland, liver, soft tissue, etc.) Our findings demonstrated increase frequency of high expression of DLL3 in Merkel cell metastases (14 of 23; 61%) versus skin primary tumors (11 of 28; 39%). There was no significant difference in frequency of high DLL3 staining intensity between primary tumors and metastases. We also compared DLL3 expression in ten cases where we had blocks from primary skin tumors, involved lymph nodes, and distant metastatic tissue, which demonstrated an increase in the frequency of high DLL3 expression in lymph nodes (9 of 15; 60%) and distant metastases (5 of 8; 63%) versus their original primary skin tumors (4 of 10; 40%). No specific parameter reached statistical significance (Fischer exact test). These results suggest that DLL3 has differential expression in metastases versus primary tumors, which deserves further study. More rigorous grading, such as using H-scoring, might demonstrate larger differences in these categories that reach statistical significance. Given the low prevalence of Merkel cell carcinoma, institutional collaboration may be required to adequately power future studies.

Poster Category: Cancer --18

Anthony Cardillo, MD
Resident: PGY-2

Fractal Compression: An Old Technique for the New Challenge of Whole Slide Image Storage

Anthony Cardillo, MD

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Background
Fractal image compression is a lossy image compression algorithm based on the idea that images often contain self-similarity. Fractal image compression can achieve extremely high compression ratios beyond its main competitor format, JPEG – at the cost of high computational complexity during the compression stage. Whole Slide Images (WSI) present a massive storage challenge – a single slide is represented on the order of gigabytes. It would be favorable to trade-off this large storage requirement for a one-time complex computational compression algorithm. The computational complexity of fractal compression is still out of reach for most desktop computers in 2020. As a surrogate, tiled histology from WSIs can act as a proof of concept to determine the self-similarity and compression ratios that could be achieved in histology.

Methods
A single 1024x1024 pixel tile from an uncompressed whole slide image of lung alveoli was exported into a lossless PNG file. Compression was performed with through three pipelines: JPEG at 90% quality, JPEG at 70%, and custom fractal code through a custom fractal compressor. The final size of all files were recorded.

Results
The lossless compressed PNG file (1.05 Megapixels) took 2,708 kilobytes of memory. The lossy JPEG file at 90% quality took 569 kilobytes of memory. At 70% quality, the JPEG file took 303 kilobytes of memory. The compressed image saved as optimized fractal code (*.ff) was 164 kilobytes, 3.5x smaller than the JPEG file at 90% quality with no subjective loss in histologic detail. Additionally, it was 1.8x smaller than the JPEG file at 70% quality.

Conclusions
Fractal compression may find a strong use-case in storage of whole-slide images – where the one-off trade for computational complexity results in massive reduction of storage size.

Bennett Wilson, DO

PGY – 4 Chief Resident

Negative Urine Cytology and the Rate of Diagnostic Agreement between Cytotechnologist and Cytopathologist: Why Not Let Cytotechnologists Sign-Out Negative Urines?

Bennett L. Wilson, DO; Shaine Babjeck, MS, CT (ASCP); Ellen J. Giampoli, MD

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Background: The Cytotechnologist's role and degree of autonomy is evolving with cytologic volume and complexity changes. We explore the possibility of cytotechnologist sign out of negative urine specimens.

Design: Following IRB approval, Cytotechnologist (CT) and Cytopathologist (CP) interpretations of voided urine (VU) and bladder wash (BW) specimens from 2013-2014 (pre-adoption of Paris System of Reporting Urine Cytology) and between 2016-2017 (post- adoption of Paris System) were compared. Patient history and ancillary studies (cystoscopy findings, FISH results, biopsy and surrounding cytology) were recorded for discrepant cases.

Results: Of 1600 cases, CT interpreted 1352 as negative, 138 as atypical, eight as suspicious for low grade urothelial carcinoma (LGUC) or LGUC, 14 as suspicious for high grade urothelial carcinoma (HGUC), 26 as HGUC, and 62 as inadequate. Of 1352 negative interpretations, a CP did not diagnose a suspicious HGUC or HGUC (*Table 1*). Overall agreement between CT and CP was 98.6% on negative cases. Viewed separately pre-Paris agreement was 99.6%, post-Paris 97.9%, VU 99.2%, and BW 97.8%.

Of 19 discrepant cases, three had subsequent studies which revealed HGUC. The remaining 16 cases had further exam findings supporting a true negative diagnosis (*Table 2*).

Conclusions: There is a high rate of agreement about negative interpretation of urinary cytology between CT and CP. There were no cases interpreted as negative by CT and diagnosed as HGUC or suspicious for HGUC by CP. This suggests CT could sign out negative screened cases, especially VU, without review by a CP.

Results	
Total urine cytology cases reviewed: 1600	
Total urine cytology cases interpreted as negative: 1352	
Total Pre-Paris System voided urine cases (VU): 300	Total Pre-Paris System bladder wash cases (BW): 300
CT negative interpretations: 271	CT negative interpretations: 255
CT and CP discrepant interpretations: 0	CT and CP discrepant interpretations: 2
	Suspicious for LGUC: 2
CT and CP negative agreement: 100%	CT and CP negative agreement: 99.2%
Total Post-Paris System voided urine cases (VU): 500	Total Post-Paris System bladder wash cases (BW): 500
CT negative interpretations: 434	CT negative interpretations: 392
CT and CP discrepant interpretations: 5	CT and CP discrepant interpretations: 12
Atypical: 3	Atypical: 8
Inadequate: 2	Inadequate: 4
CT and CP negative agreement: 98.8%	CT and CP negative agreement: 96.9%

Table 1: An overview of subcategories of specimens and discrepancy by type

Subcategory	Cytopathologist diagnosis	Previous cytology	Subsequent cytology	Biopsy	FISH	Cystoscopy
VU 2016-2018	Atypical					Negative
	Atypical	Negative x 3	Negative x 3		Negative	Negative
	Atypical	Negative x 3	Negative x 2			Negative
	Inadequate	Negative x 3				Negative
	Inadequate	No recent				Negative
BW 2013-2014	Susp. for LG		Negative x 2			Negative
	Susp. for LG					Negative
BW 2016-2018	Atypical					Negative
	Atypical	Atypical x2	Negative x 3	Negative	Inconclusive	Negative x 2
	Atypical					Negative
	Atypical		Negative			Negative
	Atypical	Negative x 2				Negative
	Atypical			**HGUC (cystectomy)**		
	Atypical	Atypical	Atypical x 2	**HG CIS**	**Positive (pre-2016)**	Negative
	Inadequate			**HGUC**	**HGUC**	**Bladder wall tumor**
	Inadequate	Negative x 3	Inadequate			Negative
	Inadequate					Trabeculation
	Inadequate	Inadequate x 2 Negative x 1	Negative x 3			Negative
	Inadequate	Negative x 3	Negative x 3		Negative	"Non-suspicious"

Table 2: Patient history and ancillary studies

Pathology Research Day
Monday, June 7, 2021
Juried Poster Session ~ 1:30 pm
Hematology and Immunology

❖ **20 – Irene Y Chen, MD ~ PGY2**
Acute Vanishing Bile Duct Syndrome in a Patient With Systemic Lupus Erythematosus Overlapping With Drug-Induced Liver Injury and Possible Primary Sclerosing Cholangitis

❖ **21 – Alexandra M. Danakas, DO ~ Fellow ~ Cytopathology**
Comparison of Diagnostic Utility of Delta-Like Ligand 3 to Insulinoma-Associated protein 1 (INSM1) in Neuroendocrine Neoplasms of Lung

❖ **22 – Xiaoqin (Lucy) Liu, MD ~ PGY2**
Clinicopathologic Characterization of Primary Appendiceal Invasive Adenocarcinoma

❖ **23 – Cynthia Reyes Barron, M ~ Fellow ~ Dermatopathology**
GLUT1 Expression in Cutaneous Sebaceous Lesions Determined by Immunohistochemistry

❖ **24 – Anthony B. Cardillo, MD ~ PGY2**
In-Silico Testing: Accurately Predicting Results of the Direct Antiglobulin Test using Commonly Available Lab Data

❖ **25 – Uday K. Baliga, MS ~ PhD 2018**
Improved multi-plasmid delivery using PEG-linked bis-PNAs

❖ **26 – Kimberly Burgos Villar, MS ~ PhD 2018**
Regulation of TRAF2-Dependent Inflammatory Signaling by Small Proline Rich Protein 1A in the Myocardium

❖ **27 – Chunmo Chen ~ PhD 2019**
Phagocytosis by osteoblast cell

❖ **28 – Sophia Eliseeva ~ PhD 2021**
Infection-induced resident macrophage reprogramming in the lung

❖ **29 – Emma House, MS ~ PhD 2018**
Investigating the Role of CD4+ T Cells in Flavorings Induced Bronchiolitis Obliterans

❖ **30 – Alison Livada, MSTP ~ PhD 2020**
Hematopoietic Progenitors Reside in the Lung

❖ **31 – Vania Lopez-Ruiz ~ PhD 2019**
Ligand presentation by XNC4, a non-polymorphic MHC class I-like molecule that confers resistance to infections by non-TB mycobacteria in *Xenopus*

❖ **32 – Cooper Sailer, MS ~ PhD 2018**
Function and Fate of Exhausted CAR T cells

Sara K. Blick-Nitko, MS

Program Year: 2017

Advisor and Department: Craig Morrell, DVM, PhD, Dept. of Medicine, Aab CVRI

The Multifactorial Roles of Platelets in Uncomplicated Malaria Infection

Sara K. Blick-Nitko[1,2], Sara K. Ture[2], Joshua Munger[3,4], Xenia Schafer[3], Craig Morrell[1,2,4]

[1]Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, [2]Department of Medicine, Aab Cardiovascular Research Institute, [3]Department of Biochemistry and Biophysics, [4]Department of Microbiology and Immunology

The *Plasmodium* parasite continues to be a major public health threat, with at least 230 million annual cases worldwide. *P vivax* is the cause of uncomplicated malaria, which while typically not deadly, it is the cause of significant global morbidity and economic cost. Despite great efforts, vaccine development has proven unsuccessful and due to growing resistance to anti-malarial drugs and long-term infection complications also persist. It is therefore crucial that we continue to explore the immunopathological mechanisms of malaria infections in order to develop improved therapies to treat the disease and its associated complications. Work from our group and others has shown that platelets have complex roles in the pathogenesis of malaria – platelets can have both protective and deleterious effects, depending on the type of infection and timing in the disease course. Results of this study will provide a better understanding of mechanisms of platelet mediated immune responses in malaria. Malaria infection elicits a strong interferon gamma (IFNγ) response. IFNγ regulates host defense to intracellular pathogens and serves a protective role during blood-stage infections. IFNγ is a potent inducer of indoleamine 2,3-dioxygenase (IDO-1), the rate-limiting enzyme that catalyzes the first step in Tryptophan (Trp) metabolism in the kynurenine (Kyn) pathway. IDO-1 maintains a balance of immune responses and immune tolerance in many pathologies and the Kyn pathway has immunomodulatory properties. Tryptophan metabolism may be altered in malaria infection as a means to regulate the host immune responses, but the mechanisms remain unknown. Our platelet RNA-sequencing data from humans infected with *P vivax* and from mice infected with *P yoelii* showed increased expression of genes related to Trp metabolism, including IDO-1. The role for platelets in metabolic pathway regulation is poorly explored in general, but particularly in infectious diseases. We introduce a novel idea that platelets participate in immunometabolism during infection.

Poster Category: Hematology and Immunology ~20

Irene Y Chen, MD

Resident: PGY-2

Acute Vanishing Bile Duct Syndrome in a Patient With Systemic Lupus Erythematosus Overlapping With Drug-Induced Liver Injury and Possible Primary Sclerosing Cholangitis

Irene Y. Chen, MD, Dongwei Zhang, B. Med., Ph.D, Xiaoyan Liao*,* B. Med., Ph.D.

Department of Pathology & Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Vanishing bile duct syndrome (VBDS) is a group of acquired disorders characterized by paucity/loss of intrahepatic bile ducts. Etiologies include medications, autoimmune diseases, infection, and neoplasms. We report a unique case of acute onset rapid progressive VBDS in a 33-year-old woman with a history of systemic lupus erythematosus and lupus nephritis (class IV/V) managed with Cellcept and Medrol. She presented initially with increased nausea and vomiting, and was noted to have elevated liver enzymes. She was given intravenous ceftriaxone and metronidazole but developed jaundice and worse cholestatic pattern of liver function tests. Imaging studies revealed no biliary abnormalities or lesions. Serology was positive for anti–ds DNA but negative for ANA, anti-mitochondria, and F-actin antibodies. Liver biopsy was performed and revealed centrilobular cholestasis with nearly complete absence of intralobular bile ducts. There was mild portal inflammation, portal and periportal fibrosis, but no features of primary biliary cholangitis, primary sclerosing cholangitis (PSC), or autoimmune hepatitis. A diagnosis of VBDS was made, favoring drug induced. The patient underwent liver transplant a year later. The explant liver showed continuous VBDS with no advanced fibrosis. Interestingly, there was multifocal replacement of mediumsized bile ducts with obliterating fibrosis, a single hilar large bile duct with xanthogranulomatous inflammation, and 1 rare residual small bile duct with periductal fibrosis, findings suggestive of PSC. Acute VBDS in a lupus patient, overlapping with drug-induced injury and late-onset PSC, has not been previously reported. This unique case demonstrates the complexity of an autoimmune disease compromising the hepatobiliary system, the mechanism of which is yet to be understood.

Alexandra M. Danakas, DO

Fellow ~Cytopathology

Comparison of Diagnostic Utility of Delta-Like Ligand 3 to Insulinoma-Associated protein 1 (INSM1) in Neuroendocrine Neoplasms of Lung

Alexandra M. Danakas, DO, Moises J. Velez, MD, Chauncey Robert Syposs, DO, Sachica C. Cheris, MD, Tanupriya Agrawal, MBBS, PhD

Department of Pathology & Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Background
Delta-like ligand 3 (DLL3) is a Notch pathway ligand that has been found to be expressed on the cell surface of small cell lung carcinoma (SCLC) as well as other neuroendocrine (NE) tumor cells. DLL3 acts as an inhibitory ligand of NOTCH receptors which regulate neuroendocrine differentiation. Insulinoma-associated protein 1 (INSM1) is a zinc-finger transcription factor that is involved in the development of NE differentiation in tissues. This study aims to compare expression of DLL3 antibody to INSM1 in neuroendocrine neoplasms of lung origin.

Design
We identified 70 cases of primary lung tumors from 2016-2020 including biopsy and resection specimens. These included 33 carcinoid tumors (26 typical and 7 atypical), 12 large cell neuroendocrine carcinomas (LCNEC), 16 SCLC, 2 combined small cell carcinoma and LCNEC, 3 adenocarcinomas and 4 squamous cell carcinomas (SCC). The tumors met the classification requirements detailed in the 2015 WHO Classification for thoracic tumors. Tissue sections were stained with VENTANA DLL3 clone SP347 Assay and INSM1 antibody clone A-8 from Santa Cruz Biotechnology, optimized for use on the Ventana platform. Ventana DLL3 (SP347) was optimized using the recommended staining conditions and tissue controls. Immunohistochemical expression of DLL3 and INSM1 was classified as either positive or negative.

Results
DLL3 expression was present in all SCLCs and LCNECs, including the combined neoplasms, which correlated to INSM1 expression, with the exception of one LCNEC in which INSM1 was negative. All lung neoplasms tested without NE differentiation were negative for both DLL3 and INSM1. Variations in DLL3 expression were identified among the carcinoid tumors, whereas INSM1 was positive in all typical carcinoid tumors (100%) with the exception of one atypical carcinoid tumor or 86% showing expression (Figures 1 & 2). The sensitivity of INSM1 (97%) was higher than DLL3 (83%), both markers were found to have 100% specificity for NE differentiation (Table 1).

Conclusion
The sensitivity of DLL3 is slightly lower in our cohort in comparison to that of INSM1, due to the variable expression among carcinoid tumors, though notably there have been some studies which also identified a

similar finding in INSM1 expression for carcinoid tumors. Our findings show the sensitivity of DLL3 is comparable to other frequentlyused markers of NE differentiation, synaptophysin, chromogranin and CD56. Furthermore, DLL3 shows promise in being a reliable diagnostic marker and therapeutic target for lung neuroendocrine neoplasm, predominantly in SCLC and LCC where expression was found in 100% of cases.
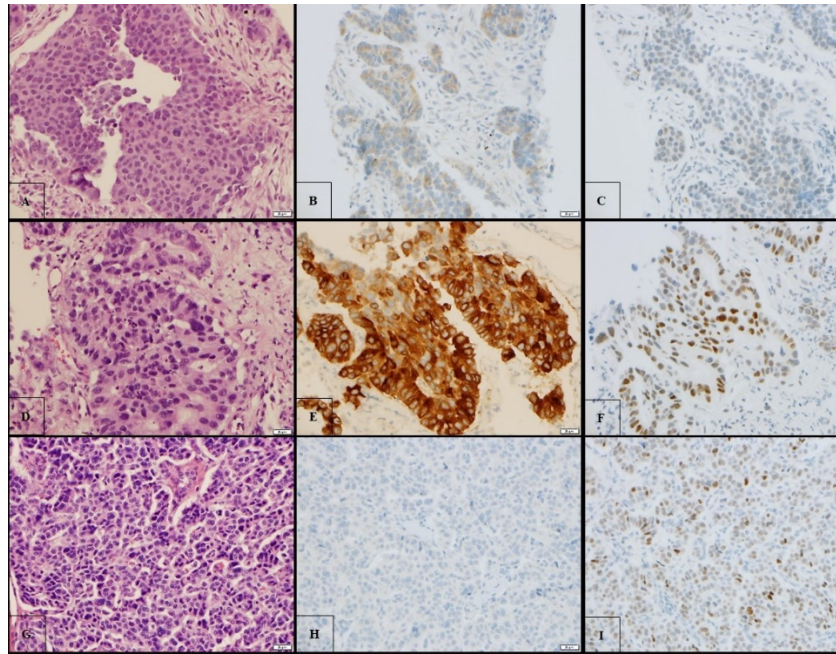

Figure 1 Legend: Atypical carcinoid tumor (H&E, A) with DLL3 expression (B) and no INSM1 expression (C); Large cell carcinoma (H&E, D) with DLL 3 (E) and INSM1 (F) expression; Typical carcinoid tumor (H&E, G) with no DLL3 expression (H) and present INSM1 expression (I). All photomicrographs taken at 400x.

Tumor Type	DLL3 and INSM1 Positive n (%)	DLL3 Positive/INSM1 Negative n (%)	DLL3 Negative/INSM1 Positive n (%)	DLL3 and INSM1 Negative n (%)
Typical Carcinoid (n=26)	19 (73)	0 (0)	7 (27)	0 (0)
Atypical Carcinoid (n=7)	3 (43)	0 (0)	3 (43)	1 (14)
Large Cell Carcinoma (n=12)	11 (92)	1 (8)	0 (0)	0 (0)
Small Cell Carcinoma (n=16)	16 (100)	0 (0)	0 (0)	0 (0)
Combined Large and Small Cell Carcinoma (n=2)	2 (100)	0 (0)	0 (0)	0 (0)
Adenocarcinoma (n=3)	0 (0)	0 (0)	0 (0)	3 (100)
Squamous Cell Carcinoma (n=4)	0 (0)	0 (0)	0 (0)	4 (100)

Figure 2 Legend: Discordant cases highlighted: No DLL3 expression (green); No expression of both DLL3 and INSM1 (blue); No INSM1 expression only (yellow).

Table 1: DLL3 and INSM1 Expression in Primary Lung Neoplasms

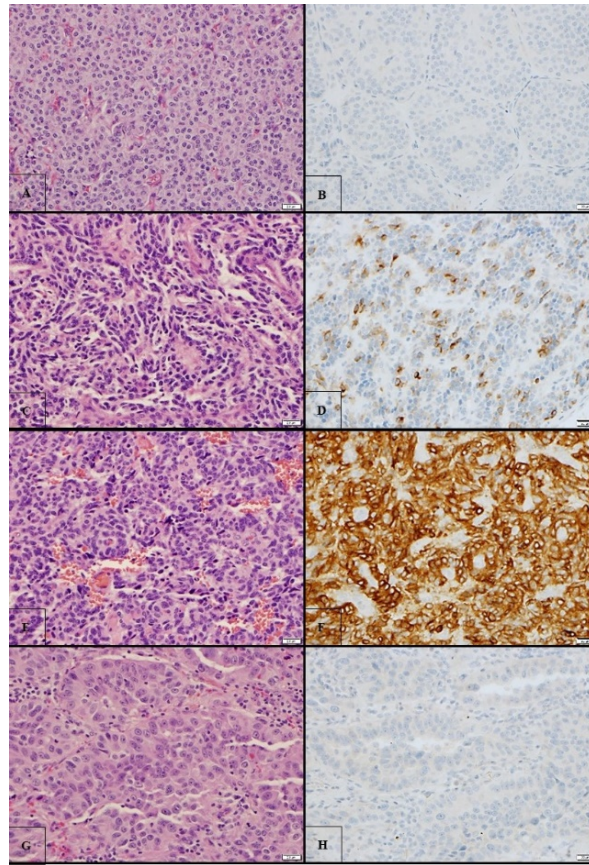| Tumor type	DLL3 Positive n (%)	DLL3 Negative n (%)	INSM1 Positive n (%)	INSM1 Negative n (%)
Typical Carcinoid (n=26)	19 (73)	7 (27)	26 (100)	0 (0)
Atypical Carcinoid (n=7)	3 (43)	4 (57)	6 (86)	1 (14)
Large Cell Carcinoma (n=12)	12 (100)	0 (0)	11 (92)	1 (8)
Small Cell Carcinoma (n=16)	16 (100)	0 (0)	16 (100)	0 (0)
Combined Large and Small Cell Carcinoma (n=2)	2 (100)	0 (0)	2 (100)	0 (0)
Adenocarcinoma (n=3)	0 (0)	3 (100)	0 (0)	3 (100)
Squamous Cell Carcinoma (n=4)	0 (0)	4 (100)	0 (0)	4 (100)
Sensitivity	83%		97%	
Specificity	100%		100%	

Figure 1 legend: Typical carcinoid tumor (H&E, A) with no DLL3 expression (B); Typical carcinoid tumor (H&E, C) with low DLL3 expression (D); Typical carcinoid tumor (H&E, E) with high DLL3 expression (F); Adenocarcinoma (H&E, G) with no DLL3 expression (H). All photomicrographs taken at 400X.

Table 1: DLL3 expression in lung primary neoplasms

Tumor Type	DLL3 Negative n (%)	DLL3 Low Expression n (%)	DLL3 High Expression n (%)
Typical Carcinoid (n=26)	7 (27)	6 (23)	13 (50)
Atypical Carcinoid (n=7)	2 (29)	1 (14)	4 (57)
Large Cell Carcinoma (n=12)	0 (0)	0 (0)	12 (100)
Small Cell Carcinoma (n=18)	0 (0)	4 (22)	14 (78)
Combined Large and Small cell carcinoma (n=2)	0 (0)	0 (0)	2 (100)
Adenocarcinoma (n=3)	3 (100)	0 (0)	0 (0)
Squamous Cell Carcinoma (n=4)	4 (100)	0 (0)	0 (0)

Xiaoqin (Lucy) Liu, MD

PGY-1

Clinicopathologic Characterization of Primary Appendiceal Invasive Adenocarcinoma

Xiaoqin Liu, MD; Irene Chen, MD; Xiaoyan Liao, MD

Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Background: Appendiceal invasive adenocarcinoma (APAC) is rare and morphologically resembles its counterpart in colon. The clinicopathologic features are not well understood.

Design: A total of 17 cases of APAC diagnosed between 2005 and 2020 were identified at our institution. In addition, 25 cases of goblet cell adenocarcinoma (GCA) and 22 cases of low-grade appendiceal mucinous neoplasm (LAMN), both at advanced tumor stages (≥T3) and diagnosed over the same period of time, were included for comparison.

Results: The APCA cohorts included 17 patients, 10 women and 7 men, with a median age of 54 (range: 28-83) years. The tumors consisted of 1 (6%) pT1, 3 (18%) pT2, and 13 (76%) pT3 and above. Lymph node and distant metastasis were present in 5 (30%) patients. Histologically, 11 (65%) APCAs were conventional adenocarcinoma resembling colorectal origin, of which 4 (24%) demonstrated focal (<50%) mucinous features, and 1 showed focal sarcomatoid and signet ring cell features. Six (35%) were mucinous adenocarcinoma (≥50% mucin), of which 1 contained signet-ring cells. All APCAs were associated with mucosal precursor lesions that were classified as tubular adenoma (n=5), tubuovillous adenoma (n=5), villous adenoma (n=2), or LAMN-Tis (n=5). Four cases had mixed mucosal adenoma and LAMN-Tis as precursor lesions. APCA with mucin production (>5% mucin) were more frequently associated with LAMN-Tis or villous adenoma (9/10, 90%), and had higher tumor stages than conventional adenocarcinoma (P<0.05). In contrast, only 2 of 25 GCA demonstrated mucosal precursor lesions (P<0.000). Compared to GCA, APCA had a higher frequency of nuclear β catenin expression (10/12 [83%] vs. 7/25 [28%], p<0.01), abnormal p53 expression (7/12 [58%] vs. 3/25 [12%], p<0.01), and SATB2 loss (5/12 [42%] vs. 2/25 [8%], P<0.05). Both GCA and APCA showed similar low frequency of SMAD4 loss and mismatch repair protein deficiency. On follow up, 4 (24%) patients died after a median survival of 20 months. Kaplan-Meier survival analysis revealed a 5-year overall survival rate of 65%. Patients with advanced (≥T3) APCA and GCA had similar overall survival, but worse than patients with LAMN beyond pTis (P<0.05).

Conclusion: This case series of APCA, the largest reported so far, demonstrated its association with various precursor lesions and distinct immunohistochemical profiles. Both APCA and GCA had worse survival than LAMN, suggesting the importance of distinguishing from the latter.

Cynthia Reyes Barron, MD

Fellow ~ Dermatopathology

GLUT1 Expression in Cutaneous Sebaceous Lesions Determined by Immunohistochemistry

Cynthia Reyes Barron, MD; Bruce R. Smoller, MD

Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

GLUT1 is a membrane associated carrier protein that functions in the physiologic transport of glucose across cell membranes. Multiple studies have shown increased GLUT1 expression in various tumor types and a possible role in cancer prognosis. The aim of this study was to determine whether cutaneous sebaceous lesions have differential expression of GLUT1 by immunohistochemistry (IHC). GLUT1 IHC was performed on excision specimens of 10 cases of sebaceous carcinoma, 9 of sebaceoma, 10 of sebaceous adenoma, and 10 of sebaceous hyperplasia. Most of the lesions were from the head and neck. Intense, diffuse cytoplasmic staining was observed in sebaceous carcinoma. Although GLUT1 is a membrane protein, the strong cytoplasmic staining seen in these neoplastic cells demonstrated the presence of the protein in high quantities beyond the membrane, within the cytoplasm. Sebaceomas and sebaceous adenomas are tumors comprised of a combination of basaloid cells and mature sebaceous cells with basaloid cells predominant in sebaceomas and mature cells in adenomas. The pattern of GLUT1 staining in these lesions consisted of a gradient of intense cytoplasmic staining in the basaloid cells with a decrease in intensity to membranous staining only and absent staining in mature sebaceous cells. The immature basaloid cells in these tumors stained like sebaceous carcinomas. As the cells appeared to mature histologically and acquired the morphology of mature sebaceous cells, the cytoplasmic staining became less intense, focal and marginal, then disappeared entirely in the most differentiated cells. In lesions of sebaceous hyperplasia, GLUT1 staining outlined the basal layer of each gland; cytoplasmic staining was minimal to absent. Increased cytoplasmic staining of GLUT1 may correlate with cellular metabolic and proliferative activity. GLUT1 has potential utility in differentiating between sebaceous lesions.

Anthony B. Cardillo, MD
Resident: PGY-2

In-Silico Testing: Accurately Predicting Results of the Direct Antiglobulin Test using Commonly Available Lab Data

Anthony B. Cardillo, MD; Chauncey Syposs, DO; Majed Refaai, MD

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Introduction
The Direct Antiglobulin Test (DAT) is a useful screening test to determine whether a patient's red blood cells have been sensitized to immunoglobulin or complement. Like the majority of screening tests, the DAT compromises specificity for sensitivity in order to quickly assess for hemolysis and its dangerous sequelae. Because of the high sensitivity of the DAT, improper ordering of this test can confuse the clinical picture at best, and result in misdiagnosis at worst. Guidelines for limiting inappropriate test ordering have proven effective in similar tests, such as the Pulmonary Embolism Rule-out Criteria ("PERC rule") for limiting the use of sensitive D-dimers. A similar pre-test could prove useful in DATs. We use "in silico testing" –testing viacomputer simulation –to predict the positivity of a DAT given common patient attributes and laboratory values.

Methods
A two-layer deep-learning artificial neural network (ANN) was created using Python and the machine learning framework Keras. The ANN was optimized to maximize specificity while retaining 100% sensitivity. Input variables to the model were patient sex and age, along with most recent lab values for hemoglobin, hematocrit, white blood cell count, platelet count, total bilirubin, direct bilirubin, lactate dehydrogenase, and haptoglobin, where available. The output of the ANN was a binary variable: "likely to be positive" versus "likely to be negative". The ANN was trained on all positive and negative DATs performed on November 2019 through March 2020, with a final total of 156 patients. Eighty percent of patient data were used for training of the networks; twenty percent of patient data were used for testing with balanced stratification.

Results
The ANN was able to maximize specificity to 25% while maintaining 100% sensitivity. A ROC curve of the model shows performance above that of the no-discrimination line with an AUROC of 0.79. Informedness using Youdin's Criteria was 0.49, or 49% increase in informedness over random guessing.

Conclusions
"In-silico testing" – the process of using computer simulations or neural networks to predict test results – can accurately predict the result of a labor and time-intensive test, such as the DAT, before the actual test is performed. This gives the ordering clinician a remote sense of the necessity of performing the test, increasing clinician informedness and potentially expediting the use of appropriate therapy (in the case of DATs, this would be systemic steroids). Analogous to their clinical counterpart "the PERC rule," computer models that maximize specificity while maintaining 100% sensitivity could minimize the number of inappropriate tests ordered and performed.

Uday K. Baliga, MS

Program Year: 2018

Advisor and Department: David Dean, PhD, Dept. of Pediatrics, Neonatology

Improved multi-plasmid delivery using PEG-linked bis-PNAs

Uday K. Baliga[1], Melissa Jagrosse[2], Bradley L. Nilsson[2], and David A. Dean[3,4,5].

Department of [1]Pathology and Laboratory Medicine, URMC; [2]Department of Chemistry, University of Rochester; [3]Department of Pediatrics, URMC

Delivery of multiple plasmids to a single cell is highly useful to many research approaches. These research approaches include induced pluripotent stem cell (iPSC) generation, viral reverse genetics, and multi-gene interactions. Methods of multi-gene expression are available but are limited by delivery efficiencies and relative expression of the genes. These limitations impose restrictions of scale and cost that hinder research in these fields. Improving the delivery of multiple genes by increasing the efficiency of cells receiving multiple plasmids expressing different genes is therefore desired. We aim to improve this by linking plasmids with a bi-bis-PNA, achieving single plasmid transfection efficiencies for co-transfection. PNAs are synthetic nucleic acids with a polyamide backbone instead of a phosphodiester backbone, this allows improved binding kinetics compared to DNA. These improved kinetics allow strand invasion in a sequence dependent manner. A bis-PNA where one PNA sequence is attached to a second PNA sequence through a short linker can further form a triplex structure or "clamp" through both Watson-Crick and Hoogsteen base-pairing of the target sequence. I use a bifunctional polyethylene glycol spacer (PEG) to link two different bis-PNAs through thioester and amine reactions creating a bi-bis-PNA. The bi-bis PNA functions as a sequence-specific method of linking multiple different plasmids together. Linked plasmid delivery only requires a single delivery event rather than multiple delivery events needing to occur simultaneously. As a result, transfection of linked plasmids should improve the co-delivery of multiple plasmids to each cell transfected compared to unlinked plasmids. I show preliminary data of plasmid linking by electrophoretic mobility shift assay (EMSA) and increased co-transfection of different fluorescent reporter plasmids when linked compared to unlinked.

Kimberly Burgos Villar, MS

Program Year: 2018

Advisor and Department: Eric Small, PhD, Dept. of Medicine, Aab CVRI

Regulation of TRAF2-Dependent Inflammatory Signaling by Small Proline Rich Protein 1A in the Myocardium

Burgos Villar KN[1,2], Burke RM[2], Small EM[2,3]

[1]Department of Pathology; [2]Aab Cardiovascular Research Institute; [3]Department of Medicine; University of Rochester School of Medicine and Dentistry, Rochester, NY

Heart failure (HF) is a leading cause of death worldwide and defined by an inability of the heart to pump sufficient blood throughout the body. HF can be induced by persistent conditions such as high blood pressure, or by acute injuries such as myocardial infarction (MI). MI is characterized by the obstruction of a coronary blood vessel resulting in ischemia. Cardiomyocytes (CMs) affected by the ischemia undergo necrosis and apoptosis. To clear the resulting cellular debris, immune cells infiltrate the area while resident fibroblasts begin to secrete an extracellular matrix-rich scar to maintain the structural integrity of the heart. This eloquent response is essential to overcoming the initial injury, and exaggeration of any particular phase can have detrimental effects. While the etiology of disease associated with high blood pressure and MI are unique, they share similar features such as hypertrophy, inflammation, and fibrosis. To better understand the process of pathological cardiac remodeling, we performed RNA-sequencing to identify transcriptional gene expression profiles associated with pathologic fates. We identified a family of small proline-rich proteins (SPRRs) that are differentially regulated in disease; *Sprr1a* is highly upregulated in response to inflammatory cytokines in CFs and CMs in the border zone (BZ) after MI. Preliminary data suggests a predicted binding site between SPRR1A and tumor necrosis factor (TNF) receptor associated factor (TRAF)2, an E3 ubiquitin ligase that acts in response to the TNF receptor at the plasma membrane to propagate inflammatory signaling. Further investigation has led us to hypothesize that SPRR1A is altering the ubiquitination status or proteasome function in CMs to halt pro-inflammatory signaling and allow for the increase in inflammation-resolution pathways to occur. Separately, SPRR1A and TRAF2 may also be playing a role in the rate of cell survival along the border of injury. We plan to determine the role of SPRR1A *in vitro* and *in vivo* to better understand the coordination of the healing response following MI.

Poster Category: Hematology and Immunology ~ 27

Chunmo Chen

Program Year: 2019

Advisor and Department: Laura Calvi, MD, Dept. of Medicine, Div. Endocrine/metabolism, Wilmot Cancer Ctr.

Phagocytosis by osteoblast cell

Chunmo Chen[1], Emily Quarato[3], Yuko Kawano[2], Allison Li[2], Hiroki Kawano[2], Laura M. Calvi.[2,4]

[1] Department of Pathology and Laboratory Medicine, University of Rochester Medical Center; [2] Wilmot cancer center, University of Rochester medical center, Rochester, United States; [3] Department of toxicology, University of Rochester medical center, Rochester, United States; [4]Dept. of Medicine, Div. Endocrine/Metabolism, URMC

Phagocytosis, is a key process immune cells use to remove apoptotic cells without inflammation. In myelodysplastic syndrome (MDS) patients, there is an increase in the apoptosis of hematopoietic cells, therefore changes in the phagocytosis of apoptotic cells might participant in the dysfunction of the bone marrow microenvironment observed in MDS. There is growing evidence that non-professional phagocytes like mesenchymal stem cells (MSCs) in MDS have increased phagocytic ability. Our preliminary data shows that mesenchymal-osteolineage cell exhibit the capacity to engulf senescent neutrophils. Therefore, we hypothesize that the osteolineage cell could participate in homeostasis via phagocytosis of apoptotic cells. The phagocytosis would alter osteoblast phenotype to further facilitate clearance of apoptotic cells.

Cells from a murine MSC line (ST2) were differentiated to osteoblastic lineage using mineralizing media, and then incubated with senescent neutrophils isolated from mice and human for 24 hours. PKH26 is used to label the neutrophils to identify and enumerate MSCs with phagocytosis capacity. Microscopic data shows that ST2-derived osteoblast could uptake one or more intact senescent neutrophils. Flow cytometry data shows around 90% of ST2-derived osteoblasts are efferocytic and 60% are highly specialized in engulfing more than one neutrophil. Senescence and apoptosis test were conducted to test post-phagocytosis performance of osteoblasts. Osteoblasts that conducted phagocytosis have lower senescence rate than treated one without phagocytosis. Osteoblasts which uptake high amount of neutrophils have increasing in apoptosis rate. These data suggest that after phagocytosis, osteoblasts would decrease their senescence rate to facilitate further phagocytosis. However, if the osteoblasts are reaching their maximum phagocytic ability, they would conduct apoptosis directly, leading to a non-inflammatory bone microenvironment.

Our analysis suggests possible mechanism of phagocytosis by non-professional phagocytes. This model could be used to understand the cooperation of cells in the bone microenvironment and how their abnormality would facilitate the MDS progression. We hope that this model would allow us to target factors, like cytokine released during clearance to facilitate phagocytosis in the malignant environment, providing novel approaches for treatment of MDS.

Poster Category: Hematology and Immunology ~ 28

Sophia Eliseeva

Program Year: 2021

Advisor and Department: Helene McMurray, PhD, Pathology and Laboratory Medicine

Infection-induced resident macrophage reprogramming in the lung

Sophia Eliseeva[1], Andrew Martin[2], Shilpi Giri[2], Alexandra Safronova[2], Samantha Kwok[2], and Felix Yarovinsky[2]

[1]Dept. of Pathology and Laboratory Medicine, [2]Center for Vaccine Biology and Immunology, University of Rochester Medical Center

Tissue-resident macrophages play a crucial part in innate immunity, tissue homeostasis, and development. They act as one of the primary defense mechanisms against intracellular pathogens. Macrophages require the effector cytokine interferon-gamma (IFN-γ) to mount the innate immune response and clear infection. The induction of IFN-γ stimulated genes leads to pathogen clearance and tissue remodeling.

Toxoplasma gondii is a parasite that infects about a third of the world's population and is well-studied as a model of IFN-γ induction. The primary site of infection is the intestine, from which the parasite invades critical organs such as the heart, brain, and lungs. We and others have shown IFN-γ-mediated tissue remodeling at the site of infection manifesting itself in the loss of tissue-resident macrophages. While the scope of tissue remodeling at the primary infection site in response to *T. gondii* has been well-studied, the effects on peripheral tissue have yet to be described.

To begin to address this question we investigated the effects of *T. gondii* infection on embryonically-seeded resident lung macrophage populations: alveolar and interstitial macrophages. We hypothesized that infection or induction of the IFN-γ response will lead to a depletion of lung resident macrophages.

To test our hypothesis we infected mice with *T. gondii* or challenged mice with recombinant IFN-γ and harvested intestine and lungs for RT-PCR, flow, and histology. Our data showed a downregulation of both interstitial and alveolar macrophage genetic markers in the lung, suggesting a depletion of these cells. We also observed a shift of these populations via flow-cytometry and immunohistochemical analyses. We propose to further study the mechanism and cellular dynamics of IFN-γ-mediated effects on lung macrophages.

Poster Category: Hematology and Immunology ~ 29

Emma House, MS

Program Year: 2018

Advisor and Department: Matthew D. McGraw, MD, Dept. of Pediatric Pulmonology

Investigating the Role of CD4+ T Cells in Flavorings Induced Bronchiolitis Obliterans

Emma House[1,2], Soyoung Kim[2], Matthew D. McGraw[2,3]

[1]Department of Pathology, [2]Department of Pediatric Pulmonology [3]Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY 14642

Bronchiolitis Obliterans (BO) is a fibrotic lung disease characterized by submucosal collagen deposition and concentric narrowing of the small airways. Progressive obstruction of the airways leads to irreversible decline in lung function accompanied by a high incidence of mortality. While historically associated with organ transplantation, recently BO has been identified as a consequence of inhalation exposure to certain flavoring chemicals, including those used in food manufacturing and e-cigarette liquids. Induction of BO is preceded by an initial insult to the airway epithelium, triggering potent inflammation and subsequent barrier dysfunction. Dysregulated inflammation disrupts epithelial repair mechanisms, scars the airway and diminishes epithelial regenerative capacity. **We hypothesize that dysfunction within the Regulatory T-cell (T_{Reg}; CD4+CD25+FoxP3+) compartment contributes to the induction and progression of flavorings induced BO**. Using the chemical flavoring Diacetyl (DA;2,3-butanedione) our lab has developed an *in-vivo* exposure system to induce BO in rats. This system has demonstrated that repeated exposure to DA results in clinical and histological signs of BO. Bronchoalveolar Lavage Fluid (BALF) from these animals shows persistent neutrophilia and protein leakage into the airway indicative of prolonged inflammation and barrier dysfunction. Utilizing flow cytometry we have begun to characterize CD4+ T-cell populations within the lungs of these animals. Data obtained from rat whole lung homogenates shows an increased proportion of T_{regs} within the lung up to 14 days following DA exposure. Preliminary *in-vitro* data suggests that signaling through Amphiregulin (AREG) and its receptor Epidermal Growth Factor Receptor (EGFR) may be critical in maintaining T_{reg} repair function in the airways after DA exposure. Current studies aim to elucidate the role of AREG/EGFR signaling in interactions between T_{regs} and the airway epithelium in promoting repair and preventing progression of fibrosis. Identifying the mechanisms involved in T_{reg} driven epithelial repair after inhalation injury is critical in the future development of novel therapeutics to improve patient outcomes.

Alison Livada, MSTP

Program Year: 2020

Advisor and Department: Craig N. Morrell, DVM, PhD, Dept. of Medicine, Aab CVRI

Hematopoietic Progenitors Reside in the Lung

Alison Livada[1,2], Daphne Pariser [3], Sara Blick-Nitko [1,2], Sara Ture [2], Craig N. Morrell [2]

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Platelets participate in immunity by initiating neutrophil recruitment, secreting antimicrobial factors, and modulating monocytes. Platelet parent cells are megakaryocytes (Mks). Historically, studies focused on Mks' platelet production in the bone marrow (BM). More recently, an invtravital microscopy study demonstrated that Mks producing platelets are also present in the lung. Subsequently, our lab characterized lung Mks' phenotype. We showed that lung Mks have constitutively higher immune markers in comparison with BM Mks. Additionally, we found that lung Mks can acquire, process and present antigen and can activate T cells during a lung bacterial response. However, lung Mks' origin remains unknown. Some evidence suggests hematopoietic stem and progenitor cells (HSPCs), the precursor pool for all blood cells, reside in the lung and can reconstitute platelet counts in thrombocytopenic mice. Whether lung HSPCs contribute to lung megakaryopoiesis is unclear.

Here, we present an initial characterization of the HSPC population in mouse lungs. Using an antibody-mediated platelet depletion model, the lung HSPCs responded to thrombocytopenia by increasing their proliferation, as measured with BrdU incorporation. To assess HSPC responses to thrombocytopenia in a disease model, we used a nonlethal, uncomplicated murine malaria infection (*Plasmodium yoelli*). *P. Yoelli*-infected mice demonstrate thrombocytopenia on days 7-14 of infection. On day 10 of infection, the BM, but not lung, HSPC responded by increasing their numbers, as measured by relative counts on flow cytometry. By day 14, both the lung and BM HSPC populations increased in number.

Additionally, we now show preliminary evidence in support of HSPC residence in the lung. In a lung HSPC reconstitution model, transferred HSPCs remained in the lung 5 days after transplant, suggesting engraftment potential.

Overall, these data suggest that lung HSPCs may have a role in responding to hematopoietic stressors. Future studies will explore this concept and its physiologic implications in health and disease.

Vania Lopez-Ruiz

Program Year: 2019

Advisor and Department: Jacques Robert, PhD, Dept. of Microbiology and Immunology

Ligand presentation by XNC4, a non-polymorphic MHC class I-like molecule that confers resistance to infections by non-TB mycobacteria in *Xenopus*

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Due to the increasing prevalence and incidences of multidrug resistance, infections caused by *Mycobacterium tuberculosis* (*Mtb*) and non-TB mycobacteria remain a significant clinical burden that would benefit from novel reliable immuno-therapies. Unlike classical MHC class I (class Ia) molecules, MHC class I-like (class Ib) molecules may not strictly require beta 2-microglobulin (*b2m*) and peptide association for surface translocation (e.g., HLA-F). XNC4 is a non-polymorphic class Ib in the amphibian *Xenopus laevis* that confers resistance against the non-TB *M. marinum* (*Mm*). *Mm* serves a model organism to study *Mtb*. *Xenopus* tadpoles are ideally suited to investigate class Ib function because they are naturally class Ia-deficient, and have prominent class Ib-interacting innate-like T cell populations and functional lungs. Given preliminary data that suggests that XNC4 binds unusually long peptides (11-15mer) reminiscent of human HLA-F, we hypothesize that **XNC4 presents unusually long mycobacteria-derived peptides in a TAP- and *b2m*-independent manner**. Accordingly, we will (1) implement mammalian and *X. laevis* cell line expression approaches of tagged recombinant XNC4 and *b2m* using a novel lentiviral-VSV hybrid vector (collaboration A. Benraiss, Neurology, URMC); (2) Examine mechanistic requirements of surface presentation by shRNA and CRISPR/Cas9-mediated loss-of-function, flow cytometry, confocal microscopy, and co-immunoprecipitation; and (3) assess T cell activation (e.g., proliferation, gene upregulation) with *Mm*-derived peptides. Elucidating the mechanism of action of XNC4 will provide a better understanding of host interaction with mycobacteria pathogens including *Mtb* and help to direct new therapeutic strategies to combat TB.
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Poster Category: Hematology and Immunology ~ 32

Cooper Sailer, MS

Program Year: 2018

Advisor and Department: Minsoo Kim, PhD

Function and Fate of Exhausted CAR T cells

Cooper Sailer[1], Minsoo Kim[1,2]

[1]Pathology and Laboratory Medicine, URMC, Rochester, NY; [2] Department of Microbiology and Immunology, Center for Vaccine Biology and Immunology, URMC, Rochester, NY

CD8 T cells responding to chronic infections or tumor antigens undergo substantial functional and phenotypic changes, including impairment of effector function, elevated expression of inhibitory receptors, reduced ability to persist and eventually cell death. This dysfunctional state of T cells, known as ***"exhaustion",*** is mainly regulated by the elevated and sustained expression of transcription factors such as TOX. Tumor microenvironments employ several strategies to attenuate effective immune-mediated tumor killing by interfering with nearly every step required for the host immunity, including inducing irreversible T cell exhaustion. To study the effects of T cell exhaustion on anti-cancer effector functions of chimeric antigen receptor (CAR) T cells, we utilized an in vitro system where anti-HER2 CAR T cells were expanded with plate-bound anti-CD3 antibody to generate "exhausted" CAR T cells. We found that chronically stimulated CAR T cells dramatically upregulated PD1 (PD1^{high} CAR T cell) and migrated significantly less on ICAM1 and CXCL12 coated plates. Surprisingly, PD1^{high} CAR T cells exhibited superior killing capacity of target cancer cells. ***We hypothesize that T cell exhaustion differentially impacts T cell recruitment and anti-cancer cytotoxicity, which may lead to the limited efficacy of solid tumor CAR T cell therapy.*** I plan to study the effects of T cell exhaustion on migration of CAR T cells both *in vitro* and *in vivo* using a clinically relevant HER2-positive cancer model. We will use TOX overexpression, temporal deletion of TOX, and TOX KO mice to test our hypothesis. Both recruitment and persistence of CAR T cells will be monitored in vivo using a photoactivatable cre-lox recombinase (PA-Cre) mouse. A better understand of the factors that regulate fates of exhausted T cells is necessary to develop more effective and safe novel strategies to treat cancer patients.

Pathology Research Day

Monday, June 7, 2021

Juried Poster Session ~ 3:00 pm

Cell Biology and Genetics

Zoomlink: https://urmc.zoom.us/j/95151858315 POSTER SESSIONS

❖ **33 ~ Irene Y Chen, MD ~ PGY-2**
Clinicopathologic and Immunohistochemical Characterization of Primary Gastrointestinal Tract Neuroendocrine Carcinomas

❖ **34 ~ Cynthia Reyes Barron, MD ~ Fellow ~ Dermatopathology**
Amplification of Chromosome 17 Centromere and Copy Number Alterations by Microarray Comparative Genomic Hybridization in a HER2 Negative Breast Cancer Case

❖ **35 ~ Courtney Kellogg, MS ~ PhD 2016**
Multiple Roles of the Notch Ligand Jagged1 during Sensory Development of the Cochlea

❖ **35 ~ Olivia J. Marola, MS ~ PhD 2016**
Determining the cell-specific mechanisms by which the endothelin system causes glaucomatous retinal ganglion cell loss

❖ **37 ~ Jiman Han, MS ~ PhD 2017**
Investigating the lysosomal degradation and autophagy dysfunction in CLN3 disease RPE

❖ **38 ~ David Richardson, MS, MSTP ~ PhD 2017**
Walking Changes the Neural Response of Proactive and Reactive Cognitive Control

❖ **39 ~ Anthony Emanuel ~ PhD 2018**
Investigating the role of extracellular matrix elastic modulus on RPE adherence and function in our patient derived iPSC-PEG hydrogel model

❖ **40 ~ Rachel Piselli, MS ~ PhD 2018**
Cell Fate Analysis of a Novel Stem Cell & Role of the SoxE Transcription Factor in D.mel Smooth Muscle Morphogenesis

❖ **41 ~ Carol Deaton, MS, MSTP ~PhD ~ 2019**
Presenilin 1 Modulates Lysosome Function and Tau Degradation

❖ **42 ~ Trae Carroll ~PhD ~2020**
Mitochondrial Network Disruption by AD-relevant Tau Post Translational Modifications

Poster Category: Cell Biology and Genetics ~ 0

Felicia A. Gilels, MS

Program Year: 2016

Advisor and Department: Amy E. Kiernan, PhD, Department of Ophthalmology

The role of JAG1-mediated Notch signaling in the maintenance and function of cochlear sensory cells

Felicia A. Gilels[1,2], Jun Wang[1], Ph.D., Patricia White[3], Ph.D., Anwen Bullen[4] Ph.D., and Amy Kiernan[2], Ph.D.

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The mammalian inner ear consists of six sensory organs that mediate hearing and balance. Inner ear sensory regions are comprised of two major cell types, mechanosensory hair cells and their associated supporting cells. The Notch signaling pathway plays multiple essential roles in the embryonic development of inner ear sensory regions, including the establishment of the sensory progenitors as well as the decision to become either a hair cell or a supporting cell. Despite these important early roles, there is a limited understanding of Notch function postnatally, when the sensory regions are still immature. Uniquely, the Notch ligand JAG1, becomes localized to supporting cells during embryonic sensory differentiation and continues to be expressed in supporting cells postnatally and into adulthood, suggesting it may have some role in cochlear maturation or maintenance. To investigate the role of JAG1-Notch signaling in the postnatal cochlea we utilize a Cre/loxP recombination system to conditionally delete JAG1 in supporting cells at postnatal day (P)0/1 an assess for effects on hearing and cochlear morphology. Results of these studies indicate that JAG1 signaling is required postnatally for normal hearing function, as $Sox2^{CreER/+}Jag1^{fl/fl}$ mutant mice display a specific form of hearing loss termed auditory neuropathy, that specifically affects the inner hair cell pathway. Surprisingly, morphological analysis of the cochlea did not reveal cellular loss, cell fate changes, or synaptic defects substantial enough to cause the hearing loss observed in the $Sox2^{CreER/+}Jag1^{fl/fl}$ mutant mice. To further understand the molecular consequences of JAG1 deletion, RNA-seq analysis was performed at P6. Pathway analysis of $Sox2^{CreER/+}Jag1^{fl/fl}$ cochleae identified *Diaph3*, a gene involved in auditory neuropathy and hair cell stereocilia integrity, as significantly upregulated. Interestingly, scanning electron microscopy of the sensory regions in $Sox2^{CreER/+}Jag1^{fl/fl}$ revealed similar stereocilia defects as those observed in *Diaph3* gain-of-function mouse models. Taken together, our results demonstrate that JAG1 signaling in postnatal cochlear supporting cells is essential for normal cochlear function and indicates a novel role for JAG1/Notch signaling in stereocilia integrity; further understanding postnatal Notch signaling will reveal important insights into cochlear maturation.

Poster Category: Cell Biology and Genetics ~ 33

Irene Y Chen, MD,

Resident: PGY-2

Clinicopathologic and Immunohistochemical Characterization of Primary Gastrointestinal Tract Neuroendocrine Carcinomas

Irene Y. Chen, Dongwei Zhang, B.Med., Moises Velez, MD, Sierra Kovar, Xiaoyan Liao, B.Med., Ph.D.

Department of Pathology & Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Background
Primary gastrointestinal tract neuroendocrine carcinomas (GI-NECs) are malignant neoplasms that can be divided into small cell NEC (SCNEC), large cell NEC (LCNEC), and mixed adenoneuroendocrine carcinoma (MANEC) when both components are high grade and comprise ≥30% of the neoplasm. GI-NECs are rare and the clinicopathologic features are not well understood.

Design
A total of 43 patients diagnosed with primary GI-NECs (21 resections, 22 biopsies) were identified and reassessed based on the World Health Organization 2019 classification and grading criteria.

Results
The cohort included 27 men and 16 women, with a median age of 66 years. Six (14%) patients had a history of inflammatory bowel disease. Histologically, 19 (44%) were LCNEC, 12 (28%) SCNEC, and 4 (9%) combined SC/LC NEC. Eight (19%) were MANECs, in which the neuroendocrine components were all LCNEC. Tumor sites included colon (n=14, 33%), rectum (n=13, 30%), esophagus (n=9, 21%), stomach (n=3), small bowel (n=2) and ileocolonic junction (n=2). Immunohistochemically, INSM1 was the most sensitive marker for neuroendocrine expression, detected in all 28 (100%) tested cases, followed by synaptophysin (40/43, 93%), CD56 (22/35, 63%), and chromogranin (18/40, 45%). SATB2, CDX2, CK20 and CK7 were positive in 21/26 (81%), 26/37 (70%), 11/35 (31%), and 10/35 (29%) cases, respectively. Three of 25 cases (11%) were mismatch repair deficient with loss of MLH1/PMS2. The expression of transcription factors, neuroendocrine and cytokeratin markers were not associated with a specific tumor location or NEC type. Molecular analysis showed 4/6 (67%) cases harbored *TP53*, 2 (33%) *KRAS,* and 2 (33%) *PTEN* mutations. Of 21 resected tumors, 19 (90%) were pT3 and above, while 13 (62%) had nodal metastasis. Ten cases had biopsy-proven liver metastasis. Treatment modalities included chemotherapy (20 NEC-based/10 adenocarcinoma-based, 70%), radiation (n=6, 14%), or immunomodulators (n=8, 19%). On follow-up, 26 (60%) patients died after a median survival of 9 months. By Kaplan-Meier analysis, the 5-year survival rate was 20% and the prognosis was tumor stage dependent ($P<0.05$). NECs in colon had slightly better survival than other sites, while age, sex, tumor type and treatment were not significant prognostic factors.

Conclusion
Primary GI-NECs are aggressive neoplasms with poor prognosis associated with tumor stage and location. INSM1, synaptophysin, and SATB2 are sensitive markers, but not site or tumor type specific.

Poster Category: Cell Biology and Genetics ~ 34

Cynthia Reyes Barron, MD

Fellow ~ Dermatopathology

Amplification of Chromosome 17 Centromere and Copy Number Alterations by Microarray Comparative Genomic Hybridization in a HER2 Negative Breast Cancer Case

Cynthia Reyes Barron, MD; David G. Hicks, MD; M Anwar Iqbal, PhD

Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

Human epidermal growth factor receptor 2 (HER2) over-expression/amplification status is important for breast cancer treatment guidance. Fluorescence in situ hybridization (FISH) studies, with dual HER2 and chromosome 17 centromere (CEP17) probes, help determine status and incorporate HER2:CEP17 copy number ratio. We present the case of a female patient with multifocal high grade invasive ductal carcinoma (figure A, 200x) with equivocal HER2 status by immunohistochemistry. FISH analysis revealed 13.2 CEP17 signals with 2.6 HER2 signals for HER2:CEP17 ratio of 0.2 (figure B). Although the case was non-amplified for HER2, the unusually high CEP17 FISH results prompted further genomic studies. DNA microarray comparative genomic hybridization (aCGH) experiments were performed on International Standard Cytogenetic Array 4 x 44K v2.0 platform featuring approximately 43,102 custom oligonucleotides (60mers) targeting 231 regions and genome-wide coverage with 75 Kb resolution (Agilent Technologies, USA). Results revealed significant amplification of the CEP17 centromere region, confirming the FISH findings. No copy number alterations affecting the HER2 gene (*ERBB2*) were observed. However, there were significant gains and/or amplifications in chromosomes 1, 3, 6, 10, 12, 16, 17, and 20 with losses in 15, 16, 17, and 18. Chromosomes 8 and 11 had chromothripsis. Copy number alterations were seen in 54.5% of autosomal chromosomes (approximately 13.6% of genome) (figure C). Although CEP17 gains are frequently seen in breast cancer, amplifications, particularly as high as our case, are rare. Additional aCGH studies identified evidence for high copy number alteration burden, which may correlate with high tumor grade and poor prognosis.
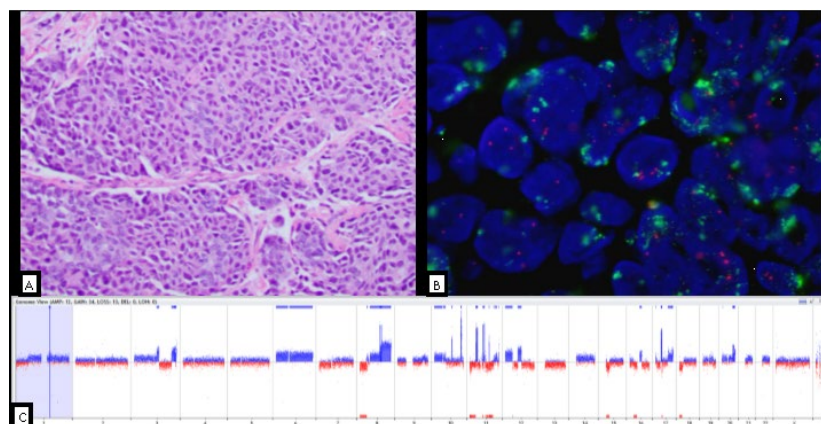
Poster Category: Cell Biology and Genetics: 35

Courtney Kellogg, MS

Program Year: 2016

Advisor and Department: Amy Kiernan, PhD, Department of Ophthalmology

Multiple Roles of the Notch Ligand Jagged1 during Sensory Development of the Cochlea

Courtney Kellogg[1,2] and Amy Kiernan[2]

[1]Department of Pathology, University of Rochester, Rochester, NY; [2]Department of Ophthalmology, University of Rochester, Rochester, NY

Hearing loss is a prevalent health issue affecting millions of individuals. Most hearing loss is caused by damage to the organ of Corti, the sensory region of the cochlea. Unfortunately, once damage occurs to critical cell types within the organ of Corti, including hair cells (HC), supporting cells (SC), and neurons, these cells cannot be regenerated or repaired. Understanding the genetic and molecular factors involved in the development of these critical cell types will aid future studies on the regeneration and/or repair of these cells. Studies have shown the Notch ligand, *Jagged1* (JAG1)*,* is critical for sensory formation in the inner ear. However, JAG1 has a very dynamic expression pattern during inner ear development, suggesting *Jag1* may have multiple roles. Here, we investigated two possible roles of JAG1: an early role in sensory progenitor development (~E9.5 & E12.5) and a later role in boundary formation (E14.5). During early otic development (~E9.5, E12.5), JAG1 is widely expressed in the sensory region of the organ of Corti. Later in development (E14.5), JAG1 becomes localized to the boundary between the inner hair cell (IHC) and outer hair cell (OHC) regions. To dissect the potential roles of JAG1 during cochlear development, we conditionally deleted *Jag1* at several developmental time points via *Foxg1+/Cre* (~E9.5), and *Sox2+/CreER* (E12.5 & E14.5) to determine when and how JAG1 is required for sensory development using marker analysis on frozen sections and wholemounts. Upon conditional deletion of *Jag1* during early sensory development (~E9.5, E12.5) there was an overall loss of sensory cells, particularly OHCs, but there were regions of the cochlea that showed excess IHCs. In addition to the loss of the sensory hair cells, there was also a significant loss in the surrounding SC. Taken together, our results support the early role of *Jag1*in sensory progenitor development. In contrast, when *Jag1* is conditionally knockout out at E14.5, there is no loss of sensory cells but the increase in IHCs was still observed. These data indicate that the increase in IHCs is due to deletion of JAG1 at later time points whereas the loss of OHCs is caused by the earlier deletion of JAG1. Along with the increase of IHC, there was no significant increase or loss in the adjacent SC, suggesting *Jag1* is plays a role in preventing further IHC development but not SC development. This increase in IHCs may be due to a disruption in boundary formation at E14.5, whereas the loss of sensory cells is likely a disruption of the early prosensory role of JAG1. In conclusion, these data support there are multiple roles of JAG1 that are separated in time during cochlear development.

Determining the cell-specific mechanisms by which the endothelin system causes glaucomatous retinal ganglion cell loss

Olivia J. Marola[1,2,3], Stephanie B. Syc-Mazurek[4], Gareth R. Howell[5], Richard T. Libby[1,3,6]

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Glaucoma is a leading cause of blindness characterized by death of retinal ganglion cells (RGCs). There is growing evidence the endothelin (EDN) system, canonically known to regulate vasoconstriction, is an important driver of glaucomatous neurodegeneration. Components of the EDN system were upregulated prior to RGC death in an ocular hypertensive mouse model of glaucoma, intravitreal injection of EDN ligands caused RGC death, and pharmacological inhibition of the EDN system lessened RGC death in mice with glaucomatous ocular hypertension. We are now taking an *in vivo* mouse genetic approach to dissect the cell-specific roles of the EDN system in glaucoma-relevant RGC death.

EDN ligands act upon EDN receptors (EDNRA and EDNRB), which are expressed by several retinal cell types—including RGCs and other retinal neurons (EDNRB), macroglia (EDNRB), and vascular smooth muscle cells (EDNRA). We first investigated whether EDN ligands act via neuronal or macroglial EDNRB to ultimately cause RGC death. *Ednrb* was conditionally deleted from retinal neurons and macroglia, EDN ligands were injected into the vitreous, and subsequent RGC death was measured. Interestingly, *Ednrb* deletion from retinal neurons and macroglia did not prevent RGC death after EDN insult. Therefore, EDN ligands likely act via EDNRA expressed by vascular smooth muscle cells to ultimately cause RGC death.

EDN ligands act through vascular EDNRA to elicit potent vasoconstriction. EDN injection caused substantial loss of retinal vascular blood flow and RGC hypoxia, which could feasibly lead to RGC death. To test this, *Ednra* was removed specifically from smooth muscle cells, EDN was intravitreally injected, and retinal vasoconstriction and RGC death were measured. Smooth muscle-specific *Ednra* deletion completely prevented both vasoconstriction and RGC death. Therefore, EDN likely insults RGCs via vasoconstriction in the context of glaucoma. Ultimately, we aim to determine the potential for targeting vascular EDNRA to prevent RGC death in glaucoma.

Poster Category: Cell Biology and Genetics ~ 37

Jiman Han, MS

Program Year: 2017

Advisor and Department: Ruchira Singh, PhD, Dept. of Ophthalmology

Investigating the lysosomal degradation and autophagy dysfunction in CLN3 disease RPE

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CLN3 disease, a rare neurodegenerative disorder with vision loss as its first clinical symptom, is most commonly caused by a 966bp deletion mutation in the *CLN3* gene. Furthermore, loss of photoreceptors and its underlying retinal pigment epithelium (RPE) is a prominent retinal pathology of CLN3 disease. In addition, the phagocytosis of daily shed photoreceptor outer segments (POS) by RPE cells, a cellular process essential for photoreceptor cell survival, is shown to be compromised in a mouse model (*Cln3*$^{\Delta ex1-6}$) and induced pluripotent stem cell (iPSC)-derived model carrying the common 966bp deletion mutation in CLN3. However, the molecular mechanism underlying impaired phagocytosis in CLN3 disease is unknown. Given that decreased protein degradation due to lysosomal-autophagy dysfunction has been shown in other cell type(s) in CLN3 disease, our goal was to examine whether lysosomal-autophagy dysfunction contributes to POS phagocytosis defect by impairing the digestion of ingested POS by RPE. We used patient-derived iPSC-RPE and isogenic embryonic stem cell (ESC; H9)-derived RPE harboring the 966bp *CLN3* mutation, paired with control iPSC-RPE derived from unaffected family members and ESC-RPE derived from parental H9, respectively. Mature pluripotent stem cell (PSC: ESC/iPSC)-derived RPE were challenged with physiologic level of POS (20 POS/RPE cell) for 2h. Western blot and immunocytochemical analyses were utilized to compare the rate of ingested POS degradation in control versus CLN3 PSC-RPE 24h post-POS feeding; 24h duration has been previously established as being sufficient to degrade POS in control PSC-RPE. Additionally, expression and localization of key proteins/enzymes in the lysosomal-autophagy pathway were evaluated by Western blotting and immunocytochemistry. Patient-derived PSC-RPE showed *i)* decreased rate of POS degradation, *ii)* altered expression of lysosomal proteins (LAMP1 and CTSB), and *iii)* decreased ratio of LC3II/LC3I (an indicator of autophagy function) compared to control PSC-RPE. Thus, our results support a role of impaired lysosomal-autophagy dysfunction in impaired POS processing by RPE cells in CLN3 disease. Ultimately, comprehending the molecular basis of POS processing defect in CLN3 disease will have therapeutic implications.

David Richardson, MS, MSTP

Program Year: 2017

Advisor and Department: John J Foxe, PhD and Edward G. Freedman, PhD, Dept. of Neuroscience;

Walking Changes the Neural Response of Proactive and Reactive Cognitive Control

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The sensory and motor processing required for even simple coordinated actions (like walking) can interfere with ongoing cognitive tasks. This degradation of cognitive processes could arise from disruption of planning in anticipation of the task (proactive control) and/or disruption of execution of the task (reactive control). Behavioral costs which stem from this cognitive-motor interference are worse in older than younger adults. Whereas younger adults utilize proactive control strategies during cognitive tasks, older adults shift towards reactive control modes, potentially as an adaptive measure to cope with age-related neurocognitive changes. As cognitive-motor demands are progressively increased, further adaption by older adults may be limited. Younger adults may better compensate by redistributing available reserves of cognitive resources and shifting to reactive control modes should difficulty exceed a threshold "tipping point".

In this study, cognitive-motor loads were systematically increased during cued task-switching while underlying neurophysiologic changes in proactive and reactive mechanisms were measured. Brain activity and kinematics were recorded from 40 healthy young adults, and 17 healthy older adults using (EEG) based Mobile Brain/Body Imaging (MoBI), as they sat or walked while performing cued task-switching.

Walking-dependent changes to neural responses during proactive and reactive control intervals were larger in younger adults. The effects of walking on evoked neural responses systematically increased as task difficulty was increased in younger, but not older adults. Mean task performance did not decrease during walking in either age group, though performance was higher in younger adults across task conditions.

These data suggest proactive and reactive control processes may be modified more extensively in younger in response to a simultaneous motor load. Though the significance of this is not yet clear as neither group suffered walking-induced performance costs, systematically increasing changes to the neural responses of younger adults may reflect progressive redistribution of resources as cognitive load increased.

Poster Category: Cell Biology and Genetics ~ 39

Anthony Emanuel

Program Year: 2018

Advisor and Department: Ruchira Singh, PhD, Dept. of Ophthalmology

Investigating the role of extracellular matrix elastic modulus on RPE adherence and function in our patient derived iPSC-PEG hydrogel model

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Age-related macular degeneration (AMD) and related macular dystrophies (MDs) are a major cause of vision loss with AMD being the leading cause of blindness in adults > 50 years of age. In the eye, AMD/MDs affect the outer blood-retinal barrier (oBRB) complex that is composed of retinal pigment epithelium (RPE) cells and its underlying vascular support, the choriocapillaris (CC). Notably, the acellular extracellular matrix (ECM) sandwiched between the RPE and CC, the Bruchs Membrane (BrM) is the primary site of disease pathology in AMD/MDs. However, it is currently not known whether and how BrM dysfunction contributes to AMD/MD pathology. Using ECM scaffolds and an immortal RPE cell line, ARPE19, a recent study has shown that stiffness (elastic modulus) of the ECM beneath the RPE cells modulates RPE adherence, metabolic activity, and cytokine production. Furthermore, BrM stiffness increases with age in vivo. However, the consequence of ECM stiffness for AMD/MD development is not known. Using induced pluripotent stem cells (iPSCs)- derived RPE from patients with AMD/MDs and unaffected control (no history of retinal degeneration) subjects, we and others have shown that pathological phenotypes of AMD/MD can be mimicked in vitro using patient-derived cells. However, iPSC-RPE model(s) currently do not incorporate a BrM like ECM. Here, we utilized polyethylene glycol (PEG) hydrogels incorporating integrin like peptides (RGD) as a synthetic extracellular matrix to act as a BrM scaffold for iPSC-RPE in our model. Furthermore, we evaluated the impact of varying the elastic modulus (3, 5, 25, 50, 500 kPa) on RPE cell characteristics including morphology and pigmentation, expression and localization of RPE signature genes/proteins (e.g EZR, ZO1, MITF) and formation of functional tight junction. Our data shows that elastic modulus (stiffness) of the ECM-scaffold can independently impact RPE cell characteristics suggesting that alterations in BM modulus with age or disease influence RPE cell function and the onset of AMD/MD like phenotypes.

Poster Category: Cell Biology and Genetics ~ 40

Rachel Piselli, MS

Program Year: 2018

Advisor and Department: Benoit Biteau, PhD, Dept. of Biomedical Genetics

Cell Fate Analysis of a Novel Stem Cell & Role of the SoxE Transcription Factor in *D.mel* Smooth Muscle Morphogenesis

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Homeostasis of vascular smooth muscle is reliant on tight control of self-renewal and differentiation heterogeneous progenitor sources with a high cellular plasticity, and failure to do so results in disease. There is an urgent need to establish genetically tractable models for studying conserved mechanisms in smooth muscle progenitors. We have developed a model to address basic questions about smooth muscle in the *Drosophila melanogaster* ovary relating to highly conserved regulatory mechanisms of governing self-renewal, migration, and differentiation in somatic stem cells. Preliminary data supports that Sox100B, homologous to mammalian Sox E transcription factor, as a genetic marker for smooth muscle progenitors. We have identified a novel sheath progenitor called Peritoneal Sheath Progenitors (PSPs). Sox100B is transiently expressed in both early progenitors, although it remains unanswered if the temporal regulation of Sox100B expression is essential to regulate self-renewal and differentiation in smooth muscle precursors and what controls Sox100B expression. Preliminary functional analysis suggests the Fibroblast Growth Factor (FGF) receptor functions upstream of Sox100B expression, making a clear connection between developmental signaling pathways and transcription factor functioning in smooth muscle progenitors managing temporal proliferation and differentiation during morphogenesis. **We first hypothesize that the Sox100B acts in PSPs to control their proliferation and differentiation in a temporally controlled manner. Secondly, we propose FGF signaling is essential to control the transient Sox100B expression in smooth muscle progenitors.** Until recently, Sox E transcription factors were not thought to regulate smooth muscle progenitors, but rather a key determinate of osteogenic/chondrogenic lineage. It remains a major challenge to test genetic requirements of smooth muscle morphogenesis directly relevant to designing better therapeutic interventions for vascular disease in the smooth muscle. Work from this project will provide further *in vivo* evidence of Sox E factors regulating smooth muscle fate.

Poster Category: Cell Biology and Genetics ~ 41

Carol Deaton, MS, MSTP

Program Year: 2019

Advisor and Department: Gail V. W. Johnson, PhD, Dept. of Anesthesiology and Perioperative Med.

Presenilin 1 Modulates Lysosome Function and Tau Degradation

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Introduction: A classical hallmark of Alzheimer's disease (AD) is the accumulation of tau, which is in part likely due to dysfunction of lysosome-dependent degradative pathways. Interestingly, the majority of autosomal dominant familial AD (FAD) cases are caused by mutations in Presenilin 1 (PS1). PS1 is classically known as the catalytic subunit of the γ-secretase complex, but more recently, it has been observed to facilitate lysosomal function and vacuolar flux. We hypothesize that, in neurons, PS1 mediates lysosome function, localization, and fusion events, and, thus, dysfunction or depletion will negatively impact tau clearance. **Methods:** Endogenous PS1 was knocked down in mature rat primary cortical neurons using shRNA-PS1 lentivirus; scramble lentivirus (SCR) served as the control. We also over-expressed mutant human PS1 in primary cortical rat neurons and human embryonic kidney cells. Multiple assays were performed to evaluate vacuolar activity and protein turnover. **Results:** PS1 depletion reduced signal of LysoTracker® DND99, a marker of acidic compartments. Taken together with a decrease in lysosome marker, Lamp2a, this may indicate fewer and/or poorly acidified lysosomes relative to SCR. Concomitantly, decreased abundance and maturation of lysosomal protease, cathepsin L, in PS1-depleted samples was quantified by immunoblot. Similarly, reduced Cathepsin D puncta assessed with Pepstatin A BODIPY™ FL Conjugate via live-cell imaging, indicated a possible reduction in degradative lysosomes or their hydrolases; quantification pending. According to immunoblot analysis, autophagosome marker, LC3BII, was decreased post 8 days treatment with shPS1, which may indicate reduced biogenesis of autophagosomes. However, at 12 days of PS1 depletion, LC3BII seemed to increase, which may indicate aggregation of autophagosomes that could not undergo terminal degradation post lysosome fusion. Importantly, we also demonstrated increases in AD-relevant phosphorylated species of tau in PS1-depleted samples relative to SCR despite no difference in total tau levels. Preliminary immunoblot analyses of LRP1, a putative cell-surface receptor for tau, may indicate that increases in tau levels may be due to altered uptake and/or release of tau. **Conclusion:** Our data suggest that PS1 dysfunction/loss of function could contribute to the evolution of tau pathology in AD, and furthermore, this may be due to dysregulation of vacuolar-mediated tau clearance.

Poster Category: Cell Biology and Genetics ~ 42

Trae Carroll

Program Year: 2020

Advisor and Department: Helene McMurray, PhD, Dept. of Pathology and Laboratory Medicine

Mitochondrial Network Disruption by AD-relevant Tau Post Translational Modifications

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The pathology of neurodegenerative disorders like Alzheimer's Disease (AD) frequently involves the accumulation of misfolded tau protein. Various pathogenic post-translational modifications (PTMs) of tau, like phosphorylation at Threonine 231 (T231) are tightly linked with AD neurodegeneration. Recent studies by Guha et al. have shown that phosphorylation of T231 impacts mitochondrial remodeling by hindering ROS-induced mitophagy, and it is thought that this disruption could impact cellular proteostasis and contribute to the early stages of neurodegeneration observed in tauopathies like AD.

To further study the impact that phosphorylation of T231E has on neuronal mitochondria, a single allele of human 0N4R tau was introduced into *C. elegans* using CRISPR-Cas9 and was exclusively expressed in the 6 mechanosensory neurons under the mec7 promoter. Single-copy mutant strains of tau, including T231E (to mimic permanent phosphorylation at site T231), and T231A (to mimic phosphoablation at site T231 as a control) were also generated under the mec7 promoter. A Mitochondrial Localization Sequence (MLS) fluorescent marker was also introduced to each worm strain, and mitochondrial morphology of each strain was assessed as a function of age. To assess the capacity for mitochondrial repair in the worms, strains were also treated with Paraquat to stimulate ROS generation and induce oxidative stress for 24 hours before assessment at developmental Day 3.

Unlike tau-overexpression models, single-copy tau PTMs did not create substantial differences in mitochondrial morphology at baseline; however, T231E strains experienced a significant reduction in mitochondrial area and density after treatment with Paraquat compared to wild-type and T231A controls. This phenotype worsened with age, leading to a significant reduction in mitochondrial area and density in the T231E strain at Day 10, without Paraquat induction.

This model demonstrates that even low-level expression of pathogenic PTM tau can disrupt mitochondrial networking in neurons, and that this effect worsens with age or oxidative stress. These observations could provide a promising new line of inquiry into the mechanisms underlying early-stage AD development.

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