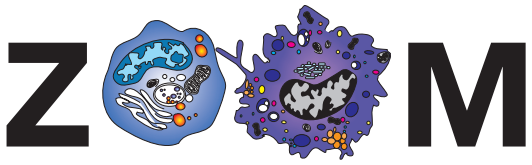
19th Annual Pathology Research Symposium

November 6, 2020

Hosted by the
Molecular and Cellular Pathology
Graduate Student Council
via



9:00am - 2:30pm

Department of Pathology

MICHIGAN MEDICINE
UNIVERSITY OF MICHIGAN

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https://www.pathology.med.umich.edu/phd-program/18th-annual-pathology-research-symposium

Welcome

It is with great pleasure that I welcome you to the 19th anniversary of the Annual Pathology Research Symposium. This annual event is organized and hosted by the Molecular and Cellular Pathology Graduate Student Council, and sponsored by the Department of Pathology at the University of Michigan.

The past eight months have been very difficult and unprecedented times for all of us in the university community as we deal with the COVID-19 pandemic. As we all are continuing to adapt and change how we live, work, and socialize, the MCP graduate program is committed and dedicated to the support of our graduate students in pursuit of their academic and professional goals. Providing platforms where the trainees can present new data, exchange ideas, and have opportunities to interact with other colleagues is an essential part of their training. Therefore, different from previous years, our students have worked actively to develop an interactive virtual meeting via Zoom.

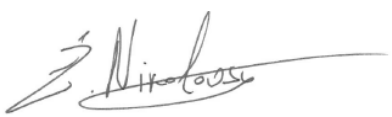
Our students have organized an exciting symposium to promote and highlight the innovation and excellence in training and research in the MCP graduate program. As in previous years, the symposium will feature an exceptional invited keynote speaker, talks and posters by our students, faculty and alumni presenting their most recent research, as well as a career panel with experts from different career paths.

We are honored to welcome the keynote speaker, Ana Maria Cuervo, M.D., Ph.D., Professor in the Departments of Development & Molecular Biology, Anatomy & Structural Biology and Medicine at Albert Einstein College of Medicine. Dr. Cuervo holds the Robert and Renée Belfer Chair for the Study of Neurodegenerative Diseases and she is co-director of the Einstein Institute for Aging Research. Dr. Cuervo is an internationally recognized physician scientist for her studies on autophagy and how malfunction of this cellular process contributes to aging, neurodegenerative and metabolic disorders. Among her early major discoveries were finding that autophagy is a highly selective process, understanding how proteins are transported into lysosomes for their degradation and how malfunctioning of the lysosomal system contributes to aging and age-related diseases. Dr. Cuervo has received numerous awards for her pioneering work and delivered prominent lectures such as the NIH Director's and the SEBBM L'Oreal-UNESCO for Women in Science. Dr. Cuervo is an elected member of the Royal Academy of Medicine of the Valencia Community, Royal Academy of Science of Spain, the American Academy of Arts and Sciences and the National Academy of Sciences.

The program also includes oral and poster presentations by our faculty and students which highlight the cutting-edge research taking place in the Department of Pathology and the MCP program. The symposium concludes with an awards ceremony presenting the 7th Annual Outstanding Research Award, as well as the best oral and poster presentations. We hope to have a high level of interactions and sharing ideas between our students, faculty and symposium participants during the oral presentations and poster discussions.

I would like to thank all the students from the MCP Student Council with special recognition to the symposium coordinators, Sahiti Marella, Jessica McAnulty, Michael Pitter and Alec Monovich. Special thanks to Dr. Jeff Rual for organizing the committee for selection of the best poster presentations, Robin Kunkel for preparing the symposium program booklet, as well as Laura Labut, the administrative coordinator of the MCP Program. Without all of their hard work, this symposium would not be possible.

Thank you for taking the time out of your busy schedules to join us and be a part of our 19th annual symposium, first conducted virtually!



Zaneta Nikolovska-Coleska, MS, PhD
Associate Professor of Pathology
Director, Molecular & Cellular Pathology PhD Program
Associate Director, Program in Biomedical Sciences (PIBS)

The University of Michigan
Graduate Program in Molecular and Cellular Pathology
presents, for the first time via ZOOM, the

19th Annual Pathology Research Symposium
Schedule of Events

November 6, 2020

9:00-9:10 am Symposium Introduction and Welcome Address

Zaneta Nikolovska-Coleska, PhD
Associate Professor of Pathology, Michigan Medicine
Director of the Molecular & Cellular Pathology Graduate Program
Faculty Associate in Interdepartmental Program in Medicinal Chemistry
Faculty Associate in the Chemical Biology Interdisciplinary Doctoral Program

9:10-9:25 am

Derek Dang, BS
Doctoral Candidate, Molecular & Cellular Pathology
Mentor: Sriram Venneti, MD, PhD
"Evaluating the role of EZHIP in posterior fossa type-A ependymomas"

9:25-9:40 am

Rita Avelar, BS
Doctoral Candidate, Molecular & Cellular Pathology
Mentor: Analisa DiFeo, PhD
"Anti-tumor Effects and Molecular Mechanisms of PP2A Reactivation for the Treatment of Ovarian Cancer"

9:40-9:55 am

David Hu, BS
Doctoral Candidate, Molecular & Cellular Pathology
Mentor: Andrew Muntean, PhD
"Characterization of the YEATS domain in MLL-ENL leukemia"

10:00-10:25am

Paul Lephart, PhD
Professor of Pathology, Inflammation & Immunology, Michigan Medicine
Kenneth and Judy Betz Family Endowed Professor
"SARS-CoV-2 Testing in the Michigan Medicine Microbiology Lab"

10:30-10:40 am **BREAK**

10:40-10:55 am

Ashwin Iyer, BS
Doctoral Candidate, Molecular & Cellular Pathology
Mentor: Russell Ryan, MD
"An 'Enhancer Hijacking' mechanism drives a subset of Diffuse Large B-Cell Lymphoma"

10:55-11:10 am

Thaddeus Kunkel, BS
Doctoral Candidate, Molecular & Cellular Pathology
Mentor: Andrew Lieberman, MD, PhD
"Myelination Deficits in Niemann-Pick Disease Type C"

11:15-11:40 am

Bernadette Zwaans, PhD *(Alumnus)*
Assistant Professor, Oakland University,
Senior Research Associate, Beaumont Health
"Modeling of Long-term Side Effects of Radiation Therapy in the Bladder"

11:40-12:30 am **BREAK**

12:30-1:30 pm **Poster Session**

1:30-2:30 pm **Keynote Presentation:**

Ana Maria Cuervo, MD, PhD

The Robert and Renee Belfer Chair
for the Study of Neurodegenerative Diseases,
Professor, Department of Developmental and Molecular Biology,
Co-Director for Aging Research,
Albert Einstein College of Medicine

"Targeting selective autophagy in aging and age-related diseases"

2:30-2:40 pm **BREAK (***official ending of symposium***)**

2:40-3:40 pm **CAREER PANEL:**

- **Ana Maria Cuervo, MD, PhD,** Albert Einstein College of Medicine
- **Bernadette Zwaans, PhD,** MCP Alumnus, Beaumont Hospital, Oakland University
- **Antonio Gomes, PhD,** Sloan Kettering Institute
- **Russel Ryan, MD,** Department of Pathology, University of Michigan Medical School

3:40-3:50 pm **AWARDS** and **Closing Remarks** to MCP Students

Panelist Biographies

Ana Maria Cuervo, MD, PhD *(Keynote Speaker)*

The Robert and Renee Belfer Chair
for the Study of Neurodegenerative Diseases,
Professor, Department of Developmental and Molecular Biology,
Co-Director for Aging Research,
Albert Einstein College of Medicine

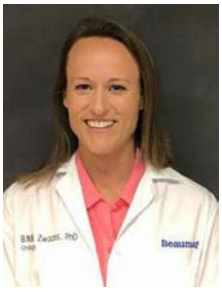
Faculty website: https://www.einstein.yu.edu/faculty/8784/ana-maria-cuervo/

Media Coverage (including The New York Times): http://www.einstein.yu.edu/faculty/experts/8784/ana-maria-cuervo/

Wiki: https://en.wikipedia.org/wiki/Ana_Maria_Cuervo

Short Bio: Dr. Ana Maria Cuervo is a Professor in Developmental and Molecular Biology, Anatomy and Structural Biology, and Medicine as well as the co-director of the Institute for Aging Studies at the Albert Einstein College of Medicine. She is also the Robert and Renee Belfer Chair for the Study of Neurodegenerative Diseases. Dr. Cuervo is considered a leader in the fields of autophagy and aging biology. Dr. Cuervo earned her M.D. and Ph.D. in molecular biology and biochemistry at the University of Valencia in Spain. In her graduate studies, she focused on lysosomal and proteosomal degradation. During her post-doctoral studies in the lab of Fred Dice at Tufts University in Boston, Dr. Cuervo made foundational discoveries in the field of chaperone-mediated autophagy (CMA), identifying the LAMP2a glycoprotein as a lysosomal receptor for this form of selective, chaperone-dependent protein degradation and importantly demonstrating that CMA declines with age. In 2001 she started her laboratory at Albert Einstein College of Medicine in New York, where she studies the role of protein-degradation and autophagy in aging and age-related disorders, with emphasis on neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's disease and metabolic disorders such as diabetes and obesity. Dr. Cuervo's work has been widely published in elite peer-reviewed journals, such as Nature and Science, and she serves as co-editor-in-chief of the journal Aging Cell. Dr. Cuervo is the recipient of numerous awards and distinctions and in 2019 was elected to the National Academy of Science.

Bernadette Zwaans, PhD *(MCP alumnus/Young Scientist)*

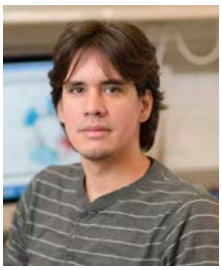


Senior Research Associate, Beaumont Health
Assistant Professor in Urology, Oakland University-
William Beaumont School of Medicine

Dr. Zwaans is senior research associate at Beaumont Health and assistant professor in Urology at Oakland University William Beaumont School of Medicine. Dr. Zwaans received her Masters in Biological Health Sciences from the University of Maastricht in the Netherlands. She subsequently worked in the department of vascular biology at Children's Hospital Boston studying the role of neuropilins in tumor angiogenesis. She then went on to receive her doctorate in Molecular and Cellular Pathology from the University of Michigan, Ann Arbor. During her doctorate training, her research focused on identifying a role for the longevity protein Sirtuin 6 in cancer. In 2015, Dr. Zwaans joined the Urology department at Beaumont Health. Based on personal experiences, she now focuses her research on improving medical care of cancer survivors. Specifically, she is studying radiation cystitis, a chronic bladder condition that is a result from pelvic radiation therapy. She has published in top journals including Cell, Urology, and Cancer Cell. She was recently awarded a prestigious K01 federal award through the NIDDK to study bladder health in cancer survivors.

Antonio Gomes, PhD *(Young Scientist)*

Computational Biologist, Senior
Memorial Sloan Kettering Cancer Center



Faculty website: https://www.mskcc.org/research/ski/labs/members/antonio-gomes

Recent original article in New England Journal of Medicine : https://www.nejm.org/doi/full/10.1056/NEJMoa1900623

Short Bio: Dr. Gomes earned a B.Sc. in Biology, a M.Sc. in Computational Biophysics from Universidade de Brasília (Brazil), Ph.D. degree in Bioinformatics from Boston University and then pursued post-doctoral studies in Systems Biology at Columbia University. He has been a computational Biologist Sr. at Memorial Sloan Kettering Cancer Center since 2017. During his undergraduate years, he developed a strong background in mathematics and was always fascinated by the intersection between mathematics and biology. During his Masters studies, he investigated how the primary sequence of a protein is capable of reversibly folding into its native, functional, 3-dimensional structure. During his Ph.D., he studied the regulatory network of Mycobacterium tuberculosis and used these data to investigate how these bacteria are able to perceive hypoxic signals in the host to switch to a latent, persistent state. Dr. Gomes also explored, via microbial dynamics and mathematical models, how resource competition could help to attenuate and treat drug resistance infection. In his post-doctoral studies, he identified a generalized mechanism that explains the regulatory compatibility among bacteria in the context of lateral gene transfer and that could have potential applications in Synthetic Biology. Currently, Dr. Gomes is investigating the role of intestinal microbiota in hematopoietic cell transplantation and its potential for therapeutic purpose.

Russel Ryan, MD *(New Principal Investigator)*

Assistant Professor
Department of Pathology, University of Michigan Medical School

Faculty website: https://www.pathology.med.umich.edu/faculty/rjhryan

Short Bio: Dr. Ryan earned a B.A. from Amherst College and M.D. from the Yale University School of Medicine. He pursued clinical training in Anatomic Pathology and Hematopathology at the Massachusetts General Hospital (MGH). He then completed a postdoctoral research fellowship in the laboratory of Bradley Bernstein at MGH & the Broad Institute. Dr. Ryan joined the faculty of the University of Michigan Medical School in 2017 as an Assistant Professor of Pathology, where he founded a research lab that focuses on mechanisms of altered gene regulation in B-cell cancers. Dr. Ryan is a practicing member of the clinical Hematopathology Service, and core member of the University of Michigan Rogel Comprehensive Cancer Center. Dr. Ryan has received research funding from the National Cancer Institute, the Leukemia and Lymphoma Society, the Leukemia Research Foundation, the V Foundation for Cancer Research, and the American Society of Hematology

ABSTRACTS
Oral Presentations

1) Evaluating the role of EZHIP in posterior fossa ependymomas

Derek Dang, Sriram Venneti

Department of Pathology and Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan, USA.

Pediatric ependymomas are the third-most common malignancy of the central nervous system. Currently, options are limited for treating these tumors. While they can arise in other areas of the neuraxis, these tumors are most commonly seen in the posterior fossa (PF). PF ependymomas have been stratified into PF-group A (PFA) and PF-group B (PFB) tumors based on transcriptional, DNA methylation and clinical profiling. PFAs carry a worse prognosis and are defined by global reduction of the repressive mark H3K27me3. PF ependymomas are unusual; as more than 80% of tumors do not bear recurrent genetic mutations. Alterations in DNA methylation and global reduction in H3K27me3 suggest that PFAs are epigenetically driven. A novel protein, EZHIP (EZH2 inhibitory protein), is expressed highly in PFAs and has been shown to drive global reduction of H3K27me3 by inhibiting histone H3K27 methyltransferase EZH2.

Our goal is to determine whether exogenous EZHIP expression can induce tumorigenicity in mouse neural stem cells. We show that EZHIP expression in these cells lowers global H3K27me3 and increases the euchromatin-inducing H3K27ac histone mark. We also demonstrate that PFAs exhibit increased expression of several key glycolytic and citric-acid cycle enzymes and metabolites. We show that exogenous expression of EZHIP in neural stem cells alter their metabolism to resemble that of PFA cells. Treatment with mitochondrial complex I inhibitor metformin increases H3K27me3 and inhibits growth in cell and *in vivo* models of PFA. Future experiments will focus on identifying key amino acid residues that may modulate interactions between EZHIP and EZH2.

2) Anti-tumor Effects and Molecular Mechanisms of PP2A Reactivation for the Treatment of Ovarian Cancer

Rita A. Avelar, Analisa DiFeo

Department of Pathology, University of Michigan Medical School

High-Grade-Serous Epithelial Ovarian Cancer (HGSOC) ranks the 5th highest cause of cancer related deaths in women and is the most common and lethal subtype of all female gynecological malignancies. HGSOC is commonly diagnosed as late-staged metastatic disease for which treatment options remain very limited. Current HGSOC treatments rely on single or combination of aggressive surgical and platinum-based chemotherapy approaches, however the success rate remains extremely poor. Therefore, there is an urgent need for the development of novel and more efficient therapies for the treatment of this disease. Targeted therapies have shown great promise in the cancer field due to their oncogenic specificity and targeting potential. Our lab investigates the anticancer properties of a first-in-class small molecule that reactivates the tumor suppressor PP2A (SMAPs), which is found to be inactive in 50% of HGSOC tumors. Our studies show that SMAPs exert potent anticancer effects in HGSOC PDX mouse models with genetically distinct backgrounds. More interestingly, our in vitro data further supports that SMAPs can specifically target tumor cells for cell death through the induction of chronic and irreversible ER stress, while sparing the non-transformed cells that can recover from these cues and restore their homeostatic balance. We also observed that SMAP activation of the PERK pathway within the UPR induces a translation arrest that is specific to cancer cells, which is irreversible and culminates in apoptotic cell death. Together our results show that the reactivation of PP2A tumor suppressor as a mean to regulate the ER stress pathway can introduce an unique and potent therapeutic opportunity for the treatment of HGSOC.

ER (Endoplasmic Reticulum)
UPR (Unfolder Protein Response)

3) Characterization of the YEATS domain in MLL-ENL leukemia

Hsiangyu Hu[1], Nirmalya Saha[1], Yuting Yang[1], Lili Chen[1], Sierrah Grigsby[1], Rolf Marschalek[2], Zaneta Nikolovska-Coleska[1], and Andrew G. Muntean[1]

[1]Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA. 48109
[2]Institute of Pharmaceutical Biology, Diagnostic Center of Acute Leukemia (DCAL), Goethe-University of Frankfurt, Frankfurt/Main, Germany. 60438

Approximately 10% of acute leukemia is driven by the MLL fusion (MLL-r) proteins. This subset of acute leukemia is characterized by deregulated activity of the SEC and the H3K79 methyltransferase DOT1L at pro-leukemic targets like HoxA9 and Meis1. The protein ENL is an MLL fusion partner/SEC component and was recently identified as an essential factor for leukemic cell growth. Importantly, the ENL epigenetic reader YEATS domain (recognizes acetylated H3K9/K18/K27) contributes to this ENL-dependency of the leukemic cells. The YEATS domain also interacts with PAF1c, an epigenetic regulator complex essential for MLL-r leukemogenesis. While these studies highlight the importance of the WT ENL YEATS domain in leukemic cells, the importance of the YEATS domain in MLL-ENL mediated leukemia remains understudied. We investigate the clinical and leukemic relevance of the YEATS domain in MLL-ENL mediated leukemogenesis. The YEATS domain is retained in 84.1% of MLL-ENL patients (n=302), and YEATS domain deletion destroys MLL-ENL leukemogenesis, increases apoptosis in cell culture, and decreases expression of pro-leukemic genes such as Meis1. Further, MLL-ENL YEATS mutants defective in interacting with PAF1 or H3Kac give rise to leukemia with significantly increased disease latency *in vivo*. Our results demonstrate an essential role for the YEATS domain in MLL-ENL mediated leukemogenesis and the importance of the YEATS domain interactions with the PAF1c or H3Kac in MLL-ENL leukemias. Together, our study establishes a rationale for small molecule development aimed at disrupting either the YEATS-H3Kac or the YEATS-PAF1 interaction for treating MLL-ENL leukemia patients.

4) An ‘enhancer hijacking’ mechanism drives a subset of Diffuse Large B-cell Lymphoma

Ashwin Iyer, Rohan Kodgule, Travis Saari, John.S.Runge, Matthew Weiss, Juhi Gupta, Niharika Rajesh, and Russell Ryan

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA

Diffuse Large B-cell Lymphoma (DLBCL) is the most common lymphoma subtype accounting for 40% of the cases in US1–3. Translocations between oncogenes such as MYC and BCL2 and the immunoglobulin loci (IG) loci are associated with inferior outcomes4–6 and drive increased oncogene expression through a mechanism known as ‘enhancer hijacking’. However, the function of translocations linking oncogenes to non-IG loci is unclear. In a subset of aggressive DLBCL cases, a recurrent t(3;8) translocation occurs between the MYC and BCL6 loci. We hypothesize that this event leads to aberrant activation of MYC by BCL6 enhancers.

To better understand the genomic architecture of the translocation, we performed Bionano optical mapping and showed that the enhancers of BCL6 are juxtaposed to the MYC gene in all three known cell lines with this translocation. We performed 4C-seq in cell lines with (n=2) and without (n=2) t(3;8) rearrangement and demonstrated topological looping of BCL6 enhancers to the promoter of MYC in t(3;8) cell lines only. Comparison of ChIP-Seq data and 4C data generated with an allele-specific primer suggested that the MYC gene is acetylated only on the t(3;8) rearranged allele.

We used CRISPR interference to target enhancer elements known to bind the BCL6-activating transcription factor (TF) MEF2B. Targeting single elements produced only a subtle reduction in growth suggesting possible compensation between enhancers. In contrast, combinatorial targeting of multiple enhancers lead to a dramatic growth reduction.
Based on this preliminary data, we plan to design and perform a high-throughput CRISPRi screen spanning the BCL6 regulatory locus to comprehensively identify essential enhancer elements. By combining this data with TF ChIP-seq, we aim to identify the regulators that sustain BCL6 enhancer activation and MYC expression.

5) Myelination Deficits in Niemann-Pick Disease Type C

Thaddeus Kunkel, Andrew Lieberman

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA

Niemann-Pick Disease Type C (NPC) is an invariably fatal disease caused by loss-of-function mutations in the NPC1 or NPC2 proteins. These proteins function to efflux cholesterol from endosomes and lysosomes; therefore, their mutation leads to the buildup of unesterified cholesterol in these compartments. NPC patients develop ataxia and cognitive decline, usually in childhood, due to neurodegeneration. Mice deficient in NPC1 recapitulate this neurodegenerative phenotype. NPC patients also show reduced myelin in white matter tracts as measured by MRI. Interestingly, NPC1-deficient mice exhibit myelin deficits prior to detectable neuron loss, suggesting a potential role for oligodendrocytes in pathogenesis. Current literature states that hypomyelination in NPC mice is due to a failure of oligodendrocyte progenitor cells (OPCs) to differentiate and mature into myelinating oligodendrocytes. This is supported by our data showing transcriptional decreases of myelin proteins and transcription factors. Our hypothesis is that dysregulation of the transcription factor SOX10 in NPC mice is responsible for stalling OPC differentiation. Our data show that SOX10 is decreased in NPC mice during critical developmental time-points. In addition to directly driving expression of myelin proteins, SOX10 also has an important influence on the epigenetic landscape. Histone modifications, specifically tri-methylation of lysine residues K27 and K9 of histone H3, play a key role in OPC differentiation. Our data indicate that these histone modifications are decreased in oligodendrocyte lineage cells of NPC mice. We are currently using primary OPC culture systems to target these pathways in an effort to increase myelin formation in the context of NPC.

ABSTRACTS
Poster Presentations

1) An enteric pathogen subverts colonization resistance by evading competition for amino acids in the gut

Gustavo Caballero-Flores[1], Joseph M. Pickard[1], Shinji Fukuda[2], Naohiro Inohara[1] & Gabriel Nunez[1]

[1]Department of Pathology and Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan, USA.
[2]Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan

Background: The gut microbiota serves as a natural barrier to prevent the invasion and expansion of pathogens, a function termed "colonization resistance". Several processes have been proposed to explain the host protection conferred by the microbiota, including production of inhibitory compounds, induction of innate and adaptive immune responses, reduction of luminal oxygen, among others. However, the strategies that bacterial pathogens employ to subvert the colonization resistance conferred by the gut microbiota are largely unknown. Results: Using a high-density mutant library generated in the enteric pathogen *Citrobacter rodentium*, we identified specific genes and metabolic pathways that the bacterium requires to colonize the gut of conventionally raised mice, but not germ-free animals. We found that *C. rodentium* amino acid biosynthesis is important for early pathogen expansion only in the presence of the microbiota. Pathogen amino acid biosynthesis pathways were induced in response to low amounts of amino acids and the presence of the gut microbiota. Reduced amounts of amino acids were found in the gut of conventionally raised mice compared with germ-free animals. Dietary administration of a high protein diet increased levels of amino acids and promoted pathogen colonization in the gut.

Conclusions: Depletion of amino acids by the microbiota limits pathogen colonization in the gut. The pathogen activates its amino acid biosynthesis pathways to overcome this nutrient deficiency and to expand in the presence of the gut microbiota. The current study may help design new strategies to treat enteric infections by targeting pathogen metabolic pathways.

2) zDHHC9-mediated palmitoylation promotes hypertrophic signaling in the heart

Kobina Essandoh[1], Arasakumar Subramani[1], Jeffery D. Molkentin[2], Matthew J. Brody[1,3]

[1]Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA.
[2]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.
[3]Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA.

Heart disease remains a leading cause of mortality, contributing to over 600,000 deaths per year in the United States. Notwithstanding, the molecular mechanisms underlying the pathophysiology of heart disease remain largely elusive. Receptors, ion channels, and signaling proteins that regulate cardiac contractility and physiology are modified by post-translational modifications such as phosphorylation. Recent research into the roles of S-acylation or palmitoylation, the reversible post-translational modification of proteins with fatty acids, has demonstrated critical functions in the regulation of intracellular signaling in physiology and disease. Palmitoylation, which is catalyzed by the zinc finger Asp-His-His-Cys (zDHHC) family of S-acyl transferases, has been implicated in trafficking, stability, sorting and binding of proteins to cofactors. However, the roles of palmitoylation in cardiac signal transduction and cardiac pathogenesis are not well understood. Our preliminary work indicates that cardiac-specific transgenic overexpression of the Golgi-specific acyl transferase zDHHC9 (TgZdhhc9) results in severe cardiomyopathy and heart failure at eight months of age in mice. Furthermore, using unbiased proteomics, we discovered that zDHHC9 palmitoylates Rab3 GTPase-activating protein catalytic subunit (Rab3gap1) that hydrolyzes the GTP-bound form of Rab3 GTPase protein (Rab3) and inactivates Rab3 function. In addition, mice subjected to transverse aortic constriction surgery, a pressure-overload model of pathological cardiac hypertrophy, showed increased palmitoylation of Rab3gap1, compared to sham controls. Altogether, these data suggest zDHHC9-mediated palmitoylation of Rab3gap1 may contribute to hypertrophic signaling and heart disease.

3) Murine iNOS expression is essential for antifungal defenses in kidneys during disseminated cryptococcosis

Kristie Goughenour[1,2*], Jessica Zaho[1,2*], Jintao Xu[1,2], Peter Zhao[2], Christine Freeman[1,2], Anutosh Ganguly[2,3], and Michal Olszewski[1,2]

[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Health System, Ann Arbor, Michigan, USA,
[2]Research Service, Ann Arbor VA Health System, Department of Veterans Affairs Health System, Ann Arbor, Michigan, USA,
[3]Department of Surgery, University of Michigan at Ann Arbor, Ann Arbor, Michigan, USA

Cryptococcus neoformans, one of the top 3 invasive fungi, causes disseminated disease leading to highly lethal outcomes. In the US, cryptococcal infections are most commonly associated with solid organ transplants. Protective immunity to cryptococcal infections is linked to a Th1 immune response and M1 macrophage polarization, which is linked to upregulation of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO), which is thought to be fungicidal. However, NO has also been linked to immune mediated pathology in other models. The role of iNOS in pulmonary cryptococcosis has been studied. Here we studied the effect of iNOS gene deletion in mouse model of disseminated cryptococcosis, which has not been characterized. We found that the infected iNOS-/- mice have significantly reduced survival times and a nearly 100-fold increase of the kidney CFU, however, no increase is seen in the lung, spleen and CNS. Histology of the kidneys revealed extensive lesions resulting in almost complete destruction of the kidney cortical area in iNOS-/- mice and the corresponding loss of kidney function (uremia) was observed. iNOS-/- mice showed increased total leukocyte recruitment, including inflammatory monocytes and neutrophils. iNOS-/- mice showed a slight bias towards M2 macrophages activation. iNOS-/- mice had no defect in T-cell polarization, but T-cell activation was somewhat decreased. Collectively, while we do not find striking differences in the systemic immunophenotype in iNOS-/- mice, we see the profound organ specific failure to control fungal growth in kidneys.

4) SIRT5 protects against pressure overload-induced heart failure via suppression of cardiac fibrosis

Angela H. Guo[1*], Rachael Baliira[2*], Mary Skinner[1], Surinder Kumar[1], Norma J. Davis[3], Sharlene Day[4], David Sinclair[5], Adam B. Stein[6+], David B. Lombard[1+]

[1]Departments of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA.
[2]Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan, MI, USA.
[3]Natural and Forensic Science Department, Albany State University, Albany, GA, USA.
[4]Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI, USA.
[5]Department of Genetics, Harvard Medical School, Boston, MA, USA.
[6]Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI, USA.

Sirtuin 5 (SIRT5) is a NAD+-dependent deacylase that removes succinyl, malonyl, and glutaryl groups from lysines to regulate proteins involved in metabolism and other pathways. Although SIRT5-deficient mice generally show very modest phenotypes, several studies have shown that these mice are more susceptible to age-associated or pathological cardiac stress. All of the studies assessing SIRT5 function in the heart have used loss-of-function approaches. Here, we generated SIRT5 overexpressing mice (SIRT5OE), and evaluated their response to chronic pressure overload (transverse aortic constriction; TAC). SIRT5OE mice showed striking protection against the adverse cardiac functional consequences of TAC compared to littermate controls. Histological and transcriptomic analysis revealed that SIRT5 suppressed fibrosis occurring in response to TAC. These results demonstrate that SIRT5 is limiting in response to chronic pressure overload.

5) Apolipoprotein E promotes immune suppression through NF-kB mediated Cxcl1 production in pancreatic cancer

Samantha B. Kemp[1], Eileen S. Carpenter[2], Zeribe C. Nwosu[3], Nina G. Steele[4], Katelyn Donahue[5], Amanda Pacheco[5], Stephanie The[6], Ashley Velez-Delgado[4], Fatima Lima7, Carlos Espinoza[7], Yaqing Zhang[7], Arvind Rao[6], Costas A. Lyssiotis[3,5,8], Marina Pasca di Magliano[4,5,7,8,*], Howard C. Crawford[3,5,8,*]

Departments of Pathology[1], Internal Medicine, Division of Gastroenterology[2], Molecular and Integrative Physiology[3], Cell and Developmental Biology[4], Cancer Biology[5], Computational Medicine and Bionformatics[6], Surgery[7], Rogel Cancer Center[8], Corresponding authors*, University of Michigan Medical School, Ann Arbor, Michigan 48109 USA.

Pancreatic cancer (PDA) is a lethal malignancy with a 5-year survival rate of only 10%. PDA is characterized by an abundant, fibroinflammatory stroma, that contains cancer-associated fibroblasts and infiltrating immune cells. Tumor-associated macrophages (TAMs) are abundant within the stroma and a key driver of immunosuppression. We and others have identified elevated expression of Apolipoprotein E (ApoE) in a subset of TAMs. Further, ApoE is elevated in human pancreatic tumors, peripheral blood, and serum protein levels stratify patient survival. ApoE has been well studied in various biological processes, but its role in pancreatic cancer has not been determined. We sought to determine whether ApoE had a functional role within the pancreatic cancer microenvironment. Based on observations in other systems, we hypothesized that it might be a mediator of immune suppression in pancreatic cancer. We implanted mouse pancreatic cancer cell lines in syngeneic wild type C57/BL6 mice or in ApoE$^{-/-}$ mice. ApoE$^{-/-}$ mice has smaller tumors compared to controls. Histological and Mass Cytometry (CyTOF) analysis revealed reprogramming in the tumor microenvironment in ApoE-/- mice. Tumors from ApoE-/- mice had an increase in CD8+ T cell and CD4+ T cell infiltration, along with a decrease in regulatory T cells. Bulk RNA sequencing of tumor cells treated with recombinant ApoE, revealed the chemokines, *Cxcl1* and *Cxcl5* are upregulated with the addition of ApoE. ApoE mediates T cell exclusion in the tumor via *Cxcl1* and *Cxcl5* production through NF-kB signaling. We are currently exploring whether ApoE loss sensitizes tumors *in vivo* to immunoregulatory agents.

6) CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy

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Cancer immunotherapy, including checkpoint blockade and adoptive T-cell transfer (ACT), results in restoration or enhancement of the cytotoxic function of tumor-specific CD8+ T cells in the tumor microenvironment. CD8+ T cells execute tumor clearance mainly by inducing cell death through perforin-granzyme- and Fas/Fas ligand-pathways. *Ferroptosis* is a form of cell death that differs from apoptosis and results from iron-dependent lipid peroxide accumulation. Although it was mechanistically illuminated *in vitro*, emerging evidence has shown that ferroptosis may be implicated in a variety of pathological scenarios. However, the involvement of ferroptosis in T cell immunity and cancer immunotherapy is unknown. Here, we find that immunotherapy-activated CD8+ T cells enhance ferroptosis-specific lipid peroxidation in tumor cells, and in turn, increased ferroptosis contributes to the anti-tumor efficacy of immunotherapy. Mechanistically, IFNγ released from CD8+ T cells downregulates expression of SLC3A2 and SLC7A11, two subunits of glutamate-cystine antiporter system xc-, restrains tumor cell cystine uptake, and as a consequence, promotes tumor cell lipid peroxidation and ferroptosis. In preclinical models, depletion of cyst(e)ine by cyst(e)inase in combination with checkpoint blockade synergistically enhances T cell-mediated anti-tumor immunity and induces tumor cell ferroptosis. Expression of glutamate-cystine antiporter system xc- is negatively associated with CD8+ T cell signature, IFNγ expression, and cancer patient outcome. Transcriptome analyses before and during nivolumab therapy reveal that clinical benefits correlate with reduced expression of SLC3A2 and increased IFNγ and CD8. Thus, T cell-promoted tumor ferroptosis is a novel anti-tumor mechanism. Targeting tumor ferroptosis pathway constitutes a therapeutic approach in combination with checkpoint blockade.

7) Anoctamin 1 (ANO1) regulates esophageal epithelial proliferation in Experimental Eosinophilic Esophagitis

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Background: Eosinophilic Esophagitis (EoE), a chronic inflammatory disease of the esophagus, is characterized by esophageal eosinophilia and epithelial remodeling, including basal zone hyperplasia (BZH) and dilated intercellular spaces (DIS). We have identified Anoctamin 1 (ANO1), a calcium activated chloride channel protein in esophageal epithelial proliferation. Herein, we employed murine models of EoE to probe the relationship between ANO1 and esophageal epithelial proliferation and EoE severity.
Methods: WT BALB/c mice were intraperitoneally injected with peanut extract (PN, 100 µg / 1mg alum), received intranasal challenges with PN (50 µg / 50 µL PBS), and oral gavaged with ground PN (2mg / 200 µL PBS). Krt5-rtTA mice backcrossed on the tetO-IL-13 background (Krt5-rtTA tetO-IL-13) received Dox (2 mg / ml) to activate esophageal IL-13 expression. EoE disease pathology (eosinophils per high power field (HPF) and BZH) was examined. Immunofluorescence (IF) analysis of murine esophagus was used to study KI-67 and ANO1 expression.
Results: PN challenge induced esophageal eosinophilia in PN-sensitized compared to control mice (Eos / HPF; 1.9 ± 0.8 vs. 7.3 ± 4.0; control vs. PN-sensitized; n = 9 per group; $p < 0.001$). IF staining revealed an increase in KI-67+ esophageal epithelial cells and robust ANO1 expression in PN-sensitized mice (KI-67+ cells / µM; 16.4 ± 1.8 vs. 21.6 ± 6.8; control vs. PN-sensitized; n = 9 per group; $p < 0.05$). Similarly, in the transgenic model, we observed increased eosinophil counts (Eos / HPF; 5.5 ± 3.5 vs. 14.0 ± 2.6; untreated vs. Dox-treated; n = 2 and 3 mice per group), KI-67+ esophageal epithelial cells (KI-67+ cells / µM; 0.08 ± 0.07 vs. 0.27 ± 0.18; untreated vs. Dox-treated; n = 3 per group) and esophageal epithelial ANO1 expression in Dox-treated versus untreated Krt5-rtTA tetO-IL-13 mice.
Conclusions: Our data reveals a relationship between esophageal epithelial proliferation and ANO1 expression in experimental EoE.

8) Amiodarone derivative induces mitotic catastrophe and apoptosis in high grade serous ovarian cancer cells

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The poor survival rates of high grade serous ovarian cancer (HGSOC) are due to a lack of preventative screening, which results in late diagnosis, and an absence of targeted therapies. Although 70% of patients initially respond to platinum-based therapy, 90% of those patients succumb to tumor recurrence and chemoresistance. There is an urgent need to develop new treatments to prolong survival of HGSOC patients. A quicker, more cost-effective, and lower-risk route to identifying new treatments is through repurposing FDA approved drugs. Using a drug repositioning platform, DrugPredict, we identified amiodarone, an antiarrhythmic agent, as a potential anti-cancer drug for ovarian cancer treatment. We found that amiodarone induced apoptosis in numerous patient-derived HGSOC cell lines, including those that were cisplatin-resistant. These effects where mediated through its ability to degrade c-MYC, which is overexpressed in >45% of HGSOC patients. Given the dose-limiting toxicity of the parent compound due to its potent hERG activity, we developed novel amiodarone derivatives that lack hERG activity but retain the anti-cancer properties and ability to regulate MYC. Our lead derivative, Compound 78, was 6-fold more potent than amiodarone, and found to be specific to ovarian cancer cells. Furthermore, unlike Amiodarone, Compound 78 induced G2/M arrest as early as 3 hours, which ultimately led to mitotic catastrophe resulting in potent cell death. In sum, these studies establish the foundation for further development of novel amiodarone derivatives and introduce a new class of therapeutics for the treatment of HGSOC and other MYC-amplified cancers.

9) Patterns of Altered Gene Expression and Chromatin Accessibility in Dermal Cells Derived from the Long-Lived Snell Dwarf Mouse

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Lower levels of GH (in mammals) and insulin/IGF1 signals in worms and flies can extend lifespan and increase resistance of mouse cells and worms to multiple forms of lethal stress. Snell dwarf mice (Pou1f1$^{dw/dw}$) feature a lifelong deficiency in growth hormone and IGF-1, and primary cells from Snell dwarf mice are resistant to multiple forms of stress, even after several weeks of expansion in tissue culture. These findings suggest that the intensity of exposure to GH and/or IGF-1 in early life may impart permanent changes in cellular traits, including enhanced stress resistance, by epigenetic means. As a first-pass survey of transcriptomic and epigenetic alterations in these stress-resistant Snell dwarf cell cultures, we have generated RNA-Seq and ATAC-Seq datasets. These data reveal broad transcriptomic alterations in these cells, including decreased expression of targets of Stat5, a major mediator of GH activity. We have also detected thousands of genomic loci with modified chromatin accessibility, most of which feature enhanced accessibility and are located far from gene promoters. Further work will be aimed at exploring the functional implications of these transcriptomic and epigenetic alterations for stress resistance and determining if these cellular signatures are present in other tissues of long-lived, GH-deficient, mutant mice.

10) C11orf95-RELA fusion aberrantly activates PI3K/Akt signaling to drive glutamine metabolism in supratentorial ependymomas

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Ependymomas are fatal brain malignancies with very few treatment options. More than 70% of supratentorial (ST) ependymomas harbor fusions of a poorly characterized gene C11orf95 with the transcriptional activator of NF-kB signaling, RELA. This oncogenic C11orf95-RELA fusion protein is shown be essential for tumorigenesis. Oncogene-driven metabolic reprogramming is a fundamental hallmark of cancer that enables sustained tumor proliferation. Our goal is to determine how this C11orf95-RELA fusion drives tumor metabolism in ST-ependymomas. To address this, we developed an in vitro isogenic system by expressing the RELA fusion protein in *Ink4a/Arf*^{null} mouse neural stem cells (RELAFUS). Using this system, we show that the cells expressing RELAFUS selectively upregulate expression of the glutamine transporter (SLC1A5) and many downstream enzymes in the glutamine metabolism pathway. Therefore, we hypothesized that the C11orf95-RELA fusion drives glutamine metabolism in ST-ependymomas. We further demonstrate that RELAFUS tumor cells utilize glutamine to maintain redox homeostasis and show marked cell death upon its withdrawal. Moreover, CB-839 and DON, both specific pharmacologic inhibitors of glutaminase, killed RELAFUS tumor cells *in vitro* and *in vivo*. Additionally, we show that these tumor cells aberrantly activate the PI3K/Akt signaling pathway by downregulating the expression of the tumor suppressor, PTEN. More importantly, the activation of PI3K/Akt signaling drives glutaminase expression and augments glutaminolysis. Our future studies include understanding how the C11orf95-RELA fusion downregulates expression of PTEN and assess the role of glutamine the pathogenesis of C11orf95-RELA fusion ependymomas. To summarize, our results suggest that the C11orf95-RELA fusion expressing tumor cells exhibit strong glutamine dependence and targeting it has significant therapeutic relevance.

11) Identification of genes of high priority in pancreatic cancer

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Genomic profiling has defined the molecular subtypes and mutational landscape of pancreatic ductal adenocarcinoma (PDAC). There is a knowledge gap on the consistency of gene expression across profiled PDAC samples and this limits the prospects of clinical translation. To facilitate novel drug target prioritization and biomarker discovery, we report the most consistently expressed genes in PDAC tissues. Across human pancreatic cancer gene expression studies, we identified ~4,000 consistent genes of which >50% are unknown. These genes largely retained their expression pattern in bulk tumor and single cell RNA sequencing data and correlated with several cancer indices, including proliferation, metastasis, mutation and tumor grade. Furthermore, ~41% predicted patients' overall survival and clustering analyses uncovered highly probable biomarkers. We identified 185 genes (notably candidates in cell cycle and glycolysis) whose knockdown suppress PDAC viability. This study represents an important milestone in the quest for mechanisms, drug targets and biomarkers in PDAC, and outlines an adaptable analytical approach that can aid discovery in other cancers.

12) Cistromically-dominant class2 mutants of FOXA1 de-repress WNT signaling to drive prostate cancer metastasis

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Forkhead box A1 (FOXA1) is a pioneer transcription factor that is essential for the normal development of several endoderm-derived organs. In the prostate gland, FOXA1 delimits tissue-specific enhancers that are activated by the androgen receptor (AR). Intriguingly, FOXA1 is frequently mutated in hormone-receptor-driven prostate, breast, bladder and salivary-gland tumors. In a recent study, leveraging an aggregate cohort of 1,546 prostate cancers, we classified FOXA1 alterations into three structural classes that diverge in clinical incidence and genetic co-alteration profiles – with a collective prevalence of 35%. Under this classification scheme, class2 mutations comprise frameshift mutations which truncate the C-terminal end of FOXA1 and are clonally acquired in the metastatic disease. Remarkably, we found truncated class2 mutants to entirely displace the wild-type FOXA1 protein from the chromatin – a characteristic we termed as "cistromic dominance" – due to higher DNA affinity. Through proteomic assays, we identified the C-terminal regulatory domain of wild-type FOXA1 to interact with, and recruit, a bonafide transcriptional WNT-repressor called TLE3 to the chromatin. Notably, class2 mutants lose this interaction and dominantly displace TLE3 from chromatin to de-repress WNT signaling. Concordantly, we found CRISPR-engineered prostate cancer cells harboring class2 FOXA1 mutations to show higher invasive and metastatic ability both *in vitro* and *in vivo*. Also, the increase in metastatic ability of class2-mutant cells was completely reversed upon treatment with a WNT inhibitor. Overall, our study reaffirms the central role of FOXA1 in mediating AR-driven oncogenesis and delineates a novel FOXA1-TLE3-WNT signaling axis that can be therapeutically co-targeted in advanced prostate cancer patients.

13) PAD4 epigenetic regulation of T-cell quiescence and its impact on anti-tumor immunity

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Studies on how epigenetic post-translational modifications regulate T-cell phenotypes offer new functional insights into adaptive immune mechanisms. Epigenetic modifications influence transcriptional programs controlling short and long-term T-cell functioning. Peptidylarginine deiminase 4 (PAD4) is an enzyme that converts arginine residues on protein targets into citrulline. PAD4 translocates into the nucleus to citrullinate histones which is sufficient to modify the chromatin landscape and influence downstream T-cell phenotype. The role of citrullination in T-cell homeostasis and polarization is understudied. Here, we show that PAD4 expression is associated with naïve and non-effector-like phenotypes. Further, we observe that PAD4 is induced by immunosuppressive cytokines such as IL10 and TGFβ. Through our work, we aim to elucidate how PAD4 expression serves T-cell biology and, importantly, how PAD4 impacts T-cell function in the tumor microenvironment.

14) Identification of novel SETDB1 regulated targets critical for leukemia proliferation

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Acute Myeloid leukemia (AML) is an aggressive hematological malignancy resulting from blockade of differentiation gene programs and enhanced self-renewal. Though advances have been made in treatment of AML, the 5 years survival rate of AML patients remains about 25%. Therefore, understanding the molecular mechanisms driving this disease is essential for the development of desperately needed novel treatments. Epigenetic modifications of DNA and histones are critical for normal hematopoietic development. Recently, we discovered that expression of SETDB1, a H3K9 methyltransferase involved in transcriptional repression, suppresses leukemogenesis in mouse AML models, which recapitulates human AML where high SETDB1 expression correlates to better prognosis. Next generation sequencing analysis in leukemic cells demonstrated expression of SETDB1 leads to repression of essential pro-leukemic targets such as *Hoxa9, Meis1*, and *Gfi1*. Importantly, our study identified a vast network of SETDB1 targets, thus presenting an opportunity to discover novel regulators of leukemogenesis. Here, using a correlation study of a CRISPR-Cas9 screen in MV411 leukemic cells we identified 63 genes as important SETDB1 targets in leukemia. We are currently evaluating the role of the 63 gene targets using a medium throughput CRISPR-cas9 based competition assay in several human leukemic cell lines. Follow up studies will focus on characterization of the importance of novel targets in a variety of human leukemia cell lines, validation in animal AML models and molecular characterization of expression control by SETDB1. Our preliminary experiments show encouraging results in our quest to identify novel gene targets important for AML maintenance.

15) Regulation of mTORC components in Snell Dwarf and GHRKO mouse livers

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mTOR (mechanistic target of rapamycin) is a kinase that can interact with other components to form two complexes, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2). These two complexes are involved in different pathways and exert diverse functions. mTORC1 is an energy sensor and controls cell growth in response to signals like growth factors and amino acids. mTORC2 functions downstream of insulin/PI3K to regulate cell proliferation and survival. A reduction of mTORC1 signaling has been shown to extend lifespan of worms, flies and mice. To study the mTOR signaling in long-lived mice, we tested the mTORC components in two mouse models, Snell Dwarf (mutation in pituitary transcription factor 1) and GHRKO (growth hormone receptor knockout), which have about 30-40% extension of their lifespan. We found a reduction of the common component DEPTOR, an increase in mTORC2 component Protor1 and changes in mTORC2 component mSin1 isoforms in livers of these mice.

16) HuR Knockout in T regulatory Cells Impairs Peripheral Tolerance

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A conditional Foxp3-Cre HuR KO mouse model was developed in order to compare the functionality of Tregs from mice lacking HuR with control Tregs from wild type mice. Gross phenotypic differences between the two strains of mice were measured using weight and hair loss. Quantitative indicators of autoimmunity were measured by glucose, protein, nitrite, and leukocyte concentration in urine, as well as levels of various cell populations in the blood. Results indicate Foxp3-Cre HuR KO mice displayed a significantly slower progression of weight gain in adults and spontaneous inflammation in multiple organs resulting in a hunched and scruffy appearance. Our results suggest mice lacking HuR RBP in regulatory T cells demonstrate impaired ability to maintain peripheral tolerance to self.

17) Regulation of IFN kappa in keratinocytes of diabetic wounds

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Chronic inflammation in non-healing wounds of Type 2 Diabetes (T2D) patients represents the most common cause of amputation and mortality. Thus, a critical need exists for understanding the wound healing defects in T2D to develop better targeted therapies. Current data points to a role for keratinocytes in orchestrating appropriate wound healing and demonstrates that wound keratinocytes in T2D are dysfunctional; however, the mechanisms that regulate this dysfunction is unclear. Interferon kappa (IFNκ), a type I IFN primarily produced by keratinocytes, has been shown to play an important role in other chronic skin diseases. Thus, this project explores the role of keratinocyte-mediated IFNκ in wound repair. To examine IFNκ expression, we utilized skin samples from T2D patients and mice, along with their respective controls. We found that IFNκ is impaired in both human and murine models of T2D wounds. This attenuation is particularly noted in basal keratinocytes. Interestingly, we identified by ChIP PCR the IFNκ promoter in T2D keratinocytes has decreased expression of H3K4me3 compared to control in a MLL-dependent manner. To further understand the role of IFNκ in wound repair, a wound curve was performed on IFNκ KO and WT mice. We show that knockout of IFNκ impairs wound healing. Thus, our data suggest IFNκ impairs wound healing in T2D patients and this attenuation of IFNκ expression in keratinocytes is regulated epigenetically. Continued investigation into the mechanism through which keratinocyte-mediated IFNκ impairs wound healing is key to development of novel treatments for T2D patients.

18) CCR2 Dependent CNS Infiltration with Inflammatory Monocytes Contributes to Fatal Immunopathology in the Brain during Cryptococcal Meningoencephalitis

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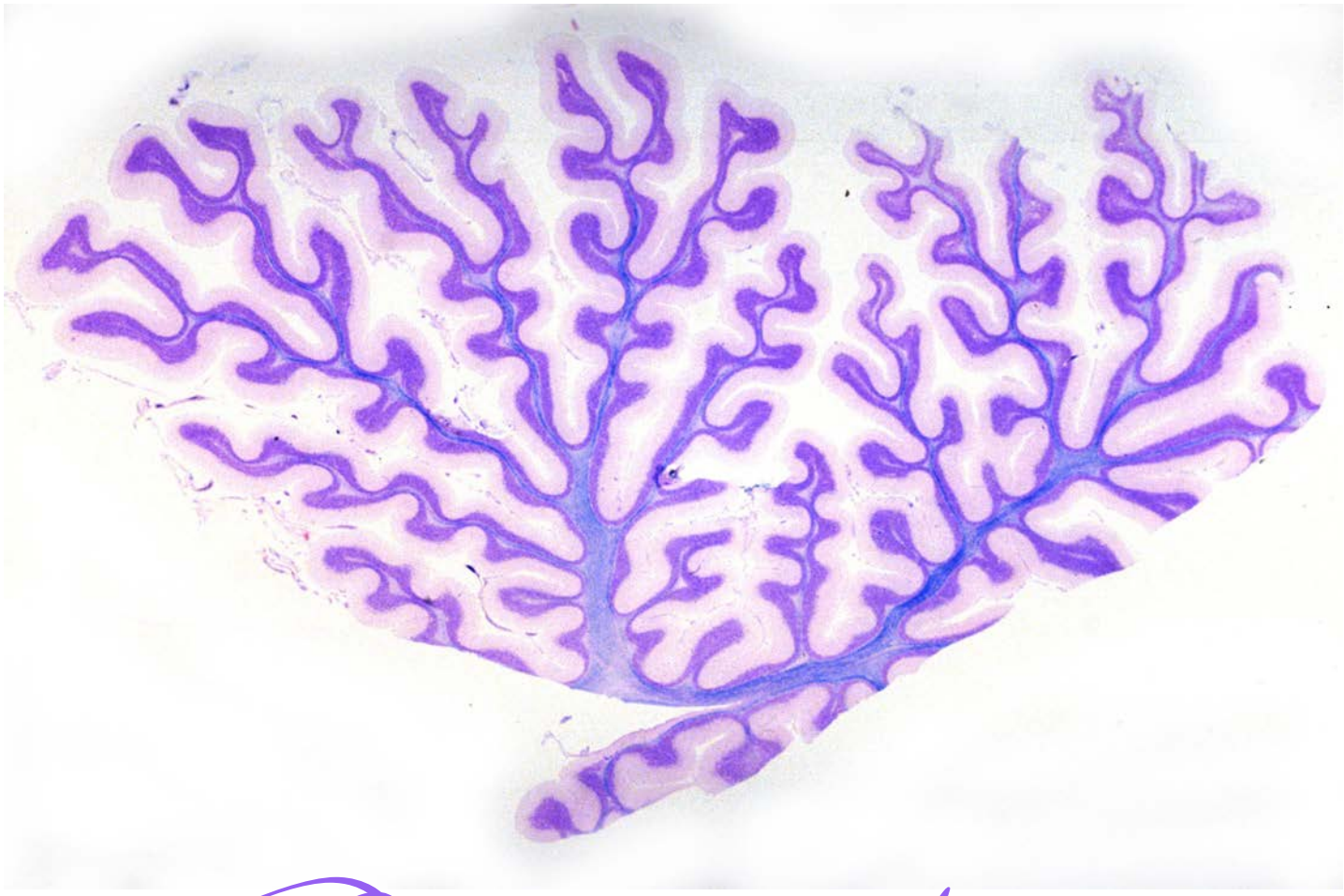
The major cause of mortality in *Cryptococcus neoformans* infection is due to the meningoencephalitis, and the pathological role of brain inflammation has become increasingly appreciated. While inflammatory monocytes (IM) are critical components of host anti-cryptococcal defense in the lungs, their roles during cryptococcal meningoencephalitis (CM) remains unclear. Using a murine model of CM, we found that IM ($CD45^{+}$ $CD11b^{+}Ly6C^{hi}Ly6G^{-}$ cells) emerged as the major myeloid population in the brain and their recruitment is dependent on C-C chemokine receptor 2 (CCR2). While CCR2 deficiency impairs IM infiltration into the brain, it markedly protects mice from fatal CM, alleviating neurological deterioration and improving the survival of animals. Improved CM outcomes in $CCR2^{-/-}$ mice are marked by reduced neuronal death and restored gene expressions in neurotransmission pathways. $CCR2^{-/-}$ mice exhibit less severe pathology despite reduced fungal clearance. Furthermore, the deletion of CCR2 prevented massive brain infiltration with pathological Th1 polarized CD4+ T cells, in favor of a more balanced but still predominantly Th1 response. Our results reveal IM are critical mediators of CM neuropathology despite their role in fungal clearance and imply that the CCR2-axis can be a potential target for neuroprotective interventions for patients with overwhelmingly inflammatory cryptococcal brain disease.



Thank You

https://www.pathology.med.umich.edu/phd-program/19th-annual-pathology-research-symposium

The Authors