

DNA Pitt Crew

The latest news and updates from the
UPMC Hillman Cancer Center Genome Stability Program



UPMC | HILLMAN
CANCER CENTER

Note from the Genome Stability Program Co-leaders, Patricia Opresko, PhD, and Bennett Van Houten, PhD

We are delighted to present the Spring 2020 edition of the DNA Pitt Crew newsletter, which provides recent information about the UPMC Hillman Cancer Center Genome Stability Program (GSP) members, new recruits, and some of the exciting scientific accomplishments from this program. The last six months have been extremely productive, and we are exceptionally proud to share highlights of recent impactful papers published in *Gastroenterology*, *Hepatology*, *Nature Chemical Biology*, *Nucleic Acids Research*, and *PNAS*, among others. Trainees and new recruits had the opportunity to share their exciting projects at our weekly work-in-progress meetings and several international meetings. We were fortunate to have several colleagues make DNA Pitt Stops in the last six months. These visits are chronicled by postdoctoral fellows and graduate students who played an important part in hosting our guests. We were especially fortunate to have Dr. Dan Durocher visit us on January 16, 2020. All of us greatly appreciated our colleagues carving out time to stop in Pittsburgh and illuminate us with their science and helpful advice. We were proud to represent our Genome Stability Program during our recent NCI Cancer Center Grant renewal on January 23, 2020. This newsletter highlights events and accomplishments prior to closures and cancellations caused by the COVID-19 pandemic. We wish everyone health and safety while we continue our scientific pursuits during this challenging time.



Faculty Spotlight – Roddy O’Sullivan, PhD

Contributed by Shanna Ridgley, Michelle Lynskey, Nicole Kaminski, and Drs. Song-My Hoang, Jonathan Barroso-Gonzalez, and Ragini Bhargava

Dr. Roddy O’Sullivan is an assistant professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh School of Medicine and a member of the Genome Stability Program at UPMC Hillman Cancer Center. Dr. O’Sullivan obtained a bachelor’s degree in genetics from Trinity College Dublin, Ireland, a doctorate in epigenetics from the Institute of Molecular Pathology in Vienna, and postdoctoral training from the Salk Institute in California, studying telomere biology under the guidance of Jan Karlseder. He joined the faculty at the University of Pittsburgh in 2014; his lab studies both the fundamental mechanisms of telomere maintenance and the clinical implications of these processes. His laboratory continues to publish highly impactful work and provides a rich training environment for graduate students and postdoctoral fellows. Recently, his first PhD student, Song-My Hoang, successfully defended her thesis. Collectively, the O’Sullivan lab describes their lab experience and boss as follows:

“Dr. O’Sullivan is perhaps the most brazen and outlandish researcher we have ever encountered. He is full of crazy ideas that always start with the catch phrase, “Wouldn’t it be cool if we...”, followed by, pretty straightforward, experimental designs. He is never afraid to push the boundaries of science...and his trainees. Dr. O’Sullivan may claim to not have a photographic memory but everyone in the GSP knows the truth.

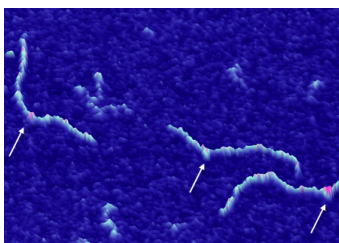
His ability to recount miniscule details regarding experimental design, results, publications/dates, and author names is uncanny. Dr. O’Sullivan’s interests include running, playing with his corgis (and children in his spare time), dining at Noodlehead, and listening to very loud music while he writes. All in all, Dr. O’Sullivan is the epitome of a well-rounded scientist and mentor. He is creative, rigorous, dedicated, and never fails to provide a good laugh at the end of the day.”

Inside this issue:

Pitt Stop: Special Events & Visiting Speakers (Page 2)
Conference Highlights & Awards (Page 5)
Hot Papers (Page 8)
Cool Science (Page 8)
Staff News (Page 10)

Contact Us:

For more information about the GSP, please contact:
Patty Opresko, PhD - plo4@pitt.edu
Ben Van Houten, PhD - vanhoutenb@upmc.edu



Atomic force microscopy (AFM) image showing XPA bound to a 538bp DNA substrate.

Trainee Spotlight — Emily Beckwitt, PhD

Contributed by Mary Byrnes and Shanna Ridgley

Congratulations to Dr. Emily Beckwitt, who defended her dissertation on November 8, 2019. Emily earned her bachelor's degree in biology: cellular and molecular biology and biochemistry from Colby College in Waterville, Maine, and continued her graduate work at the University of Pittsburgh and Carnegie Mellon University to receive her PhD, studying molecular biophysics and structural biology. Emily worked as a graduate student researcher in Dr. Ben Van Houten's lab from 2013 to 2019. She recently secured a new position as a postdoc in the lab of Dr. Mike O'Donnell at Rockefeller University in New York in January 2020. Her main research interest was in the role of XPA within damage recognition of DNA. Dr. Beckwitt is first author on two journal articles from 2017 and 2018, in addition to several others as co-author; she recently published a first author paper on XPA in *Nature Communications* (2020). She has been an invited speaker and presenter to 18 national and international conferences over the past ten years. Emily has received numerous honors and awards including an NIH T32 training grant from 2015 to 2017, a travel award from the University of Pittsburgh School of Medicine in 2016, a travel award from the former Molecular and Cellular Cancer Biology Program of the University of Pittsburgh in 2017, and the student and new investigator travel award from EMGS in 2019. The Genome Stability Program will miss Dr. Beckwitt. We wish her all the best in her new position at Rockefeller University and future endeavors.

Pitt Stop: Special Events & Visiting Speakers

Sarah Delaney, PhD

Director of Graduate Studies in Chemistry and Associate Professor, Brown University

December 6, 2019

Pittsburgh Chromatin Club Mini-Symposium

Contributed by Mary Byrnes and Shanna Ridgley

Dr. Delaney was the keynote speaker for the Pittsburgh Chromatin Club Mini-Symposium, presenting a talk titled *Overcoming the packaging paradox: DNA base excision repair in nucleosomes*. She spent some quality time with Hillman faculty and trainees to discuss her current research. Dr. Delaney is an expert in the biochemistry and enzymology of DNA repair. Her research is focused primarily on the repair of chemically-modified DNA. In addition to Dr. Delaney's keynote, other talks presented were given by Yael Nechemia-Arbely (Department of Pharmacology and Chemical Biology), *Guarding the genome: Maintaining epigenetically defined human centromeres through error correction*; Ryan Barnes (Opresko Lab), *Telomeric 8-oxo-guanine induces growth arrest in normal cells*; Huaiying Zhang (Carnegie-Mellon University), *Liquid-liquid phase separation in telomere function*; Hun-Way Hwang (Pitt School of Medicine), *Understanding alternative polyadenylation in human disease with cTag-PAPERCLIP*; and Craig Kaplan (Pitt Department of Biological Sciences), *Mechanisms of initiation by RNA polymerase II*. Stay tuned for the next Pittsburgh Chromatin Club Mini-Symposium.



Pictured left to right: Drs. Yael Nechemia-Arbely, Sarah Hengel, Sarah Delaney, and Mariarosario DeRosa, and Namrata Kumar

Pitt Stop: Special Events & Visiting Speakers



Pictured left to right: Drs. Bret Freudenthal, Patty Opresko, and Ben Van Houten

Bret Freudenthal, PhD

Assistant Professor, Department of Biochemistry and Molecular Biology, University of Kansas Medical Center
January 13-14, 2020

Contributed by Samantha Sanford (Opresko lab)

Dr. Bret Freudenthal, from the University of Kansas Medical Center, visited the DNA Pitt Crew at the beginning of January and gave both a fantastic lecture on base excision repair (BER) and a seminar on his research. Additionally, he took time to meet with trainees, during which he shared his challenges and triumphs on his career path from a postdoc to PI. On January 13, he delivered a guest lecture at the Genome Instability and Human Disease graduate course entitled *Base Excision Repair/Single Strand Break Repair*. He engaged the students' interest by first taking them through the steps of the BER pathway and then discussing various diseases caused by dysfunctional BER proteins.

On January 14, he presented his work *Mechanisms of nucleotide selection by the catalytic core of telomerase* at the UPMC Hillman Cancer Center Basic Science Seminar Series. Telomerase is a specialized reverse transcriptase that adds GGTTAG repeats to chromosome ends. Dr. Freudenthal described the ways in which his group determined the mechanism by which the telomerase catalytic active site selects canonical deoxyribonucleotides for correct telomere extension. To understand this mechanism, his group used a reductionist approach including x-ray crystallography, kinetic and molecular biology assays to evaluate this structure-function model. Dr. Freudenthal described how they used the insect model, *Tribolium castaneum*, to develop X-ray crystal structures of the TERT catalytic subunit throughout the telomerase catalytic cycle. To validate the crystal structures, his lab carefully detailed the pre-steady state kinetic analysis to understand telomerase fidelity and discrimination against ribonucleotide insertion. Finally, in collaboration with the Opresko lab, he showed us how complementary biochemical studies were done using human telomerase. This exciting work gives the telomerase field a better understanding of how telomerase can select the right versus wrong nucleotide base, and the mechanism of ribonucleotide discrimination by telomerase.



Pictured left to right: Drs. Roddy O'Sullivan, Dan Durocher, Ben Van Houten, and Patty Opresko

Dan Durocher, PhD

Senior Investigator, Lunenfeld-Tanenbaum Research Institute, Sinai Health System
January 19, 2020

Contributed by Michelle Lynskey and Nicole Kaminski (O'Sullivan lab)

Dr. Dan Durocher visited both the Oakland campus and UPMC Hillman Cancer Center in Shadyside on January 16th to speak with trainees and faculty in Pharmacology and Chemical Biology. Dr. Durocher delivered a fascinating talk entitled *Synthetic Lethality and DNA Repair Networks* that described his work in discovering proteins that are synthetically lethal together with BRCA1/2 mutations in triple negative breast cancer. Additionally, he presented an exciting project that aims to map the various genome maintenance pathways that respond to different DNA damaging cancer therapeutics. Dr. Durocher also discussed an interest in pursuing mechanisms of therapeutic resistance through analysis of differential protein expression in response to cancer treatments. With this exciting research, he and his colleagues are working towards highlighting potential synthetic lethal interactions that could provide new therapeutic strategies to target certain cancers.

Pitt Stop: Special Events & Visiting Speakers

Aishwarya (Ash) Prakash, PhD

Assistant Professor of Oncologic Sciences
University of S. Alabama

February 24-25, 2020

Contributed by Mary Byrnes and Shanna Ridgley

Dr. Ash Prakash was a guest lecturer for the Genome Instability and Human Disease Course presenting *Mismatch Repair and Cancer*. During her visit, Dr. Prakash met with several structural biology faculty members and toured the Cryo-EM Facility and NMR Facility in Oakland. In addition, she met with many GSP faculty members and trainees at UPMC Hillman Cancer Center. Dr. Prakash remarked that she enjoyed meeting the GSP postdocs and graduate students and was especially touched by their enthusiasm and passion for research. She provided career advice, and she shared her path as a young investigator. Dr. Prakash recently published in *Environmental and Molecular Mutagenesis* in October 2019; her work is titled *Mitochondrial DNA: Epigenetics and Environment*. The article, highlighted on the cover, was also selected as the editor's choice for the edition.



Pictured left to right: Sanjana Thosar, Ash Prakash, Samantha Sanford, Mariarosaria DeRosa, Namrata Kumar, Maria Beecher, Samuel Johnson, Ryan Barnes, Norie Chiba, Pinnakin Pandya, Jonathan Barosso-Gonzalez

Elise Fouquerel, PhD

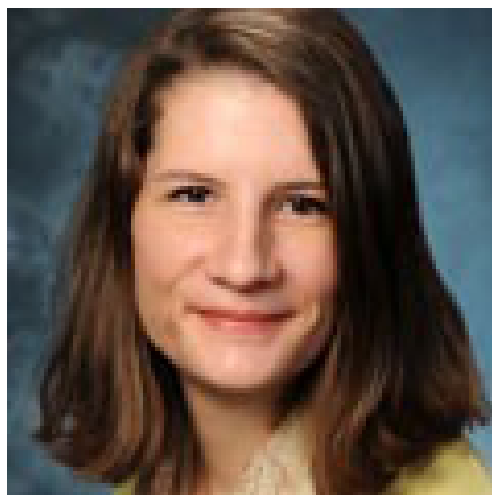
Assistant Professor in the Department of Biochemistry
and Molecular Biology

Thomas Jefferson University, Philadelphia, PA

February 27-28, 2020

Contributed by Mary Byrnes and Shanna Ridgley

Elise Fouquerel, PhD, is a former postdoc in Dr. Patty Opresko's lab who landed her first faculty position as assistant professor in the Department of Biochemistry and Molecular Biology at Thomas Jefferson University in Philadelphia in 2019. She presented *Poly(ADP)-Ribose Polymerase and PARG* as a guest lecturer for the Genome Instability and Human Disease Course on February 28, 2020. Dr. Fouquerel's research explores deciphering the roles played by Poly(ADP-ribose) polymerases (PARPs) in the maintenance of genome stability. Her research aims to provide crucial insight in understanding oxidative stress mediated tumorigenesis by uncovering the roles played by PARP in the maintenance of telomere integrity under stress conditions and in the regulation of telomerase enzyme. Her lab utilizes biochemical, molecular, and cell biology tools to address their current questions and projects. During her visit, Dr. Fouquerel met with several Genome Stability faculty members, postdocs, and graduate students working in the Opresko lab.



Dr. Elise Fouquerel

Scientific Conference Highlights and Awards

Environmental Mutagenesis and Genomics Society (EMGS) Annual Meeting in Washington, D.C.

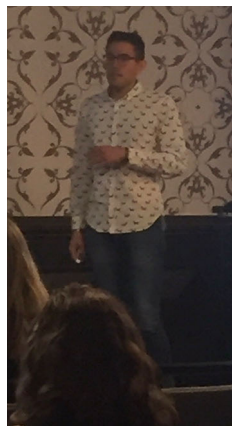
50th Anniversary

September 19-23, 2019

Several members of the Genome Stability Program attended the annual meeting in Washington, D.C. of the Environmental Mutagenesis and Genomics Society (EMGS). The meeting allowed for access to cutting-edge science as well as opportunities for networking in a way that informed, challenged, and educated.

The mini-symposium included presentations by:

- **Dr. Ben Van Houten:** *Teaching an Old Dog New Tricks: Novel Role of UV-DDB in Base Excision Repair*
- **Dr. Ryan Barnes:** *Targeted Oxidative Telomere Base Damage Induces Growth Arrest and Senescence in Normal Human Cells*
- **Dr. Emily Beckwitt:** *Single-Molecule Analysis Reveals Monomeric XPA Bends DNA and Undergoes Episodic Linear Diffusion During Damage Search*
- **Samantha Sanford:** *Investigating the Impact of Oxidized and Therapeutic dNTPs on Telomerase Activity*
- **Dr. Patty Opresko:** *Novel Precision Tools for Studying DNA Damage and Repair*
- **Namrata Kumar** presented a poster and short presentation to the DNA Repair Special Interest Group on her work: *UV-DDB: An Early Responder in the Repair of Oxidative DNA Damage*



Dr. Ryan Barnes



Samantha Sanford



Dr. Patty Opresko

Additionally, **Dr. Ryan Barnes** won second place for best platform presentation by a new investigator.

Pitt DNA Crew Traveled to Nassau, Bahamas for 4th DNA Repair/Replication Structures and Cancer Conference

Contributed by Mary Byrnes and Shanna Ridgley

Dr. Ben Van Houten and graduate students **Namrata Kumar** and **Maria Beecher** participated in the 4th DNA Repair/Replication Structures and Cancer Conference February 16-20, 2020 in Nassau, Bahamas.

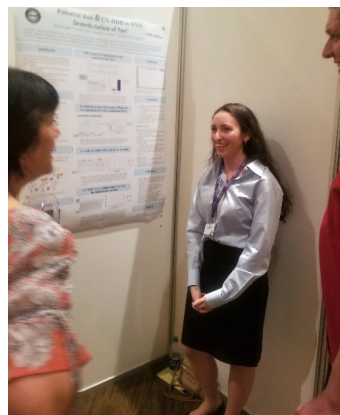
Dr. Van Houten presented a talk entitled *Watching Cooperative Interactions between Base and Nucleotide Excision Repair Proteins*.

Dr. Kara Bernstein presented a talk entitled *Novel Roles for the RAD51 Paralogs in DNA Strand Specific Damage Tolerance*.

Dr. Sunbok Jang (Van Houten lab) presented a talk entitled *Watching Cooperative Interaction Between Base and Nucleotide Excision Repair Proteins* at the Fusion Conference: DNA and Interacting Proteins as Single Molecules - In Vitro and In Vivo Conference February 20-23, 2020 in Nassau, Bahamas.

Maria Beecher, graduate student researcher (Van Houten lab) presented her poster entitled *Potential Role of UV DDB in DNA Demethylation of 5mC*; at right, Maria is pictured with Dr. Sheila David, Professor at the University of California, Davis.

Accolades to **Namrata Kumar** (Van Houten lab) for winning best poster award for her poster titled *UV-DDB is an early responder in the repair of oxidative DNA damage* at the 4th DNA Repair/Replication Structures and Cancer Conference, February 16-20, 2020 at the Melia Nassau Beach, Nassau, Bahamas.



Maria Beecher



Namrata Kumar

Scientific Conference Highlights and Awards

Congratulations, Dr. Song-My Hoang (O'Sullivan lab)

Department of Pharmacology and Chemical Biology
PhD Dissertation Defense and Final Examination

December 4, 2019

By Dr. Ben Van Houten

Dr. Song-My Hoang's work is focused on how the maintenance of telomeres is related to cancer growth. Her dissertation defense's title is *Control of Telomeric Homology-Directed Repair by Poly (ADP-ribose) Metabolism*. Dr. Hoang will continue her research as a postdoc in Dr. O'Sullivan's lab.



Congratulations, Dr. Darleny Lizardo, Postdoctoral Associate

February 3, 2020

Contributed by Dr. Lin Zhang

Congratulations to Darleny Lizardo, PhD, postdoctoral associate in Dr. Lin Zhang's lab, on receiving the 2020 AACR Minority Scholar in Cancer Research Award. This meritorious scholar award will provide her with complimentary 2020 annual meeting registration and an award check in the amount of \$1,500 to help offset expenses incurred in connection with her participation in this meeting. Darleny was born in the Dominican Republic and relocated to New York City at only ten years old. She was the first member of her family to earn a bachelor of science from Trinity College in Hartford Conn. With an unquenched thirst for knowledge, she decided to take her education a step further and enrolled at SUNY Buffalo's doctoral program in medicinal chemistry. Darleny is now a postdoctoral associate conducting research aimed at understanding the molecular mechanisms driving the interplay between cell death and immune response in microsatellite instability-high colorectal cancer. Ultimately, her goal is to lead a research laboratory at a small liberal arts college, to share her knowledge and give back.



Accolades to Dr. Ryan Barnes

March 1-6, 2020

Contributed by Dr. Ryan Barnes, Mary Byrnes, and Shanna Ridgley



Dr. Ryan Barnes, a member of Dr. Patty Opresko's lab, held a leadership role as co-chair of the Gordon Research Conference: DNA Damage, Mutation and Cancer in Ventura, Calif., on March 1-6, 2020. Dr. Barnes presented a poster titled *Oxidative base damage to telomere induces p53 dependent growth arrest*. The Gordon Research Seminar (GRS) is a meeting preceeding a Gordon Research Conference for trainees. Dr. Barnes co-organized the GRS with Judit Jimenez-Sainz of Yale University. The GRS on DNA Damage, Mutation and Cancer is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting-edge ideas through selected talks and poster presentations. This meeting fosters collaboration, networking, and community building. The focus of this meeting



was on DNA damage responses, mutagenesis processes, genetic and genomic alterations, cancer mechanisms, and targeted therapy. The keynote speaker was Dr. Aishwarya Prakash of the University of South Alabama Mitchell Cancer Institute, who also served on the mentoring panel alongside Tiago Faial from Nature Genetics, Anthony Forget from Intellia Therapeutics, Hannah Klein from the New York University School of Medicine, and Jeremy Stark from City of Hope. The mentoring component promotes networking and scientific discussion and supports career independence and diversity. Dr. Barnes and Dr. Jimenez-Sainz were able to raise almost \$8,500 for the meeting which covered registration costs of the selected speakers, discussion leaders, and mentors. The meeting was a great success with tremendous participation from the attendees. Congratulations, Dr. Barnes!

Awards and Honors

Congratulations, Dr. Ben Van Houten

Congratulations, Dr. Ben Van Houten, recipient of the UPMC Hillman Cancer Center Merrill Egorin **Excellence in Scientific Leadership Award** at the annual UPMC Employee Awards Ceremony held on October 15, 2019 at UPMC Shadyside. This award honors a faculty member that exemplifies scientific passion and scholastic dedication. It's no secret that Dr. Van Houten is a tough mentor, but his track record speaks for itself. His students are ambitious and very driven, winning poster awards at scientific conferences. The Genome Stability Program is successful and thriving thanks to the leadership provided by him. Simply put, he is the epitome of a dedicated leader and teacher. Dr. Van Houten's passion for science is contagious, and he continues to mentor very successful students. One of Dr. Van Houten's favorite phrases is "A rising tide lifts all boats" and he has worked tirelessly to advance exceptional science in the Genome Stability Program by attracting new talent, fostering the careers of junior faculty, and promoting exciting new collaborative projects.



Drs. Robert Ferris and Ben Van Houten

New Grant Awardees



Sarah Hengel



Chris Bakkenist

Sarah Hengel, postdoctoral fellow in Dr. Kara Bernstein's lab, was awarded a three-year fellowship from the American Cancer Society for her work entitled *Elucidating the role of the human RAD51 paralogs in DNA damage repair*. Dr. Hengel's work will focus on studying genes that are important for DNA repair following damage and replication stress. While over 300 epidemiology studies link human RAD51 paralog mutations to tumorigenesis, breast and ovarian cancers, few advances have been made to understand their function. Dr. Hengel is studying the function the RAD51 paralog containing complex called the Shu complex and its role in promoting error-free DNA repair.

Contributed by Sarah Hengel

Chris Bakkenist, PhD: National Cancer Institute - *DNA damage signaling to dormant origins of replication*. This grant deals with how replication origins are formed and triggered to fire during the cell cycle. Specifically, this work examines how ATR inhibitors cause firing of dormant origins.

Lin Zhang, PhD, and Jian Yu, PhD: National Cancer Institute - *Targeting defective necroptosis in colorectal cancer*. This grant deals with using genetic and pharmacological approaches to reactivate the cell death pathway, necroptosis, in cancer cells.



Lin Zhang



Jian Yu

Jian Yu, PhD, has also received a collaborative research award as part of the Development Funding Pilot Program at UPMC Hillman Cancer Center for her research focused on CRISPR-Enabled tracking of cancer cell chromosome instability and karyotype landscape evolution.

Hot Papers

- 1) M.J. Yousefzadeh, J. Zhao, C. Bukata, E.A. Wade, S.J. McGowan, L.A. Angelini, M.P. Bank, A.U. Gurkar, C.A. McGuckian, M.F. Calubag, J.I. Kato, C.E. Burd, P.D. Robbins, L.J. Niedernhofer, Tissue specificity of senescent cell accumulation during physiologic and accelerated aging of mice, [Aging Cell](#), (2020) e13094-e13094.
- 2) L. Shao, W. Hou, N.E. Scharping, F.P. Vendetti, R. Srivastava, C.N. Roy, A.V. Menk, Y. Wang, J.-M. Chauvin, P. Karukonda, S.H. Thorne, V. Hornung, H.M. Zarour, C.J. Bakkenist, G.M. Delgoffe, S.N. Sarkar, IRF1 Inhibits Antitumor Immunity through the Upregulation of PD-L1 in the Tumor Cell, [Cancer Immunol Res](#), 7 (2019) 1258-1266.
- 3) M.B. Larsen, M. Perez Verdaguer, B.F. Schmidt, M.P. Bruchez, S.C. Watkins, A. Sorkin, Generation of endogenous pH-sensitive EGF receptor and its application in high-throughput screening for proteins involved in clathrin-mediated endocytosis, [Elife](#), 8 (2019) e46135.
- 4) A.D. Singhi, L.D. Wood, E. Parks, M.S. Torbenson, M. Felsenstein, R.H. Hruban, M.N. Nikiforova, A.I. Wald, C. Kaya, Y.E. Nikiforov, L. Favazza, J. He, K. McGrath, K.E. Fasanella, R.E. Brand, A.M. Lennon, A. Furlan, A.K. Dasyam, A.H. Zureikat, H.J. Zeh, K. Lee, D.L. Bartlett, A. Slivka, Recurrent Rearrangements in PRKACA and PRKACB in Intraductal Oncocytic Papillary Neoplasms of the Pancreas and Bile Duct, [Gastroenterology](#), 158 (2020) 573-582.e572.
- 5) P.D. Wilkinson, E.R. Delgado, F. Alencastro, M.P. Leek, N. Roy, M.P. Weirich, E.C. Stahl, P.A. Otero, M.I. Chen, W.K. Brown, A.W. Duncan, The Polyploid State Restricts Hepatocyte Proliferation and Liver Regeneration in Mice, [Hepatology](#), 69 (2019) 1242-1258.
- 6) A.A. Kapralov, Q. Yang, H.H. Dar, Y.Y. Tyurina, T.S. Anthonyamuthu, R. Kim, C.M. St Croix, K. Mikulska-Ruminska, B. Liu, I.H. Shrivastava, V.A. Tyurin, H.-C. Ting, Y.L. Wu, Y. Gao, G.V. Shurin, M.A. Artyukhova, L.A. Ponomareva, P.S. Timashev, R.M. Domingues, D.A. Stoyanovsky, J.S. Greenberger, R.K. Mallampalli, I. Bahar, D.I. Gabrilovich, H. Bayir, V.E. Kagan, Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death, [Nat Chem Biol](#), 16 (2020) 278-290.
- 7) J. Martino, G.J. Brunette, J. Barroso-González, T.N. Moiseeva, C.M. Smith, C.J. Bakkenist, R.J. O'Sullivan, K.A. Bernstein, The human Shu complex functions with PDS5B and SPIDR to promote homologous recombination, [Nucleic Acids Res](#), 47 (2019) 10151-10165.
- 8) D. Bulgari, D.L. Deitcher, B.F. Schmidt, M.A. Carpenter, C. Szent-Gyorgyi, M.P. Bruchez, E.S. Levitan, Activity-evoked and spontaneous opening of synaptic fusion pores, [Proc Natl Acad Sci U S A](#), 116 (2019) 17039-17044.
- 9) T.N. Moiseeva, C. Qian, N. Sugitani, H.U. Osmanbeyoglu, C.J. Bakkenist, WEE1 kinase inhibitor AZD1775 induces CDK1 kinase-dependent origin firing in unperturbed G1- and S-phase cells, [Proc Natl Acad Sci U S A](#), 116 (2019) 23891-23893.
- 10) B. Nmezi, J. Xu, R. Fu, T.J. Armiger, G. Rodriguez-Bey, J.S. Powell, H. Ma, M. Sullivan, Y. Tu, N.Y. Chen, S.G. Young, D.B. Stolz, K.N. Dahl, Y. Liu, Q.S. Padiath, Concentric organization of A- and B-type lamins predicts their distinct roles in the spatial organization and stability of the nuclear lamina, [Proc Natl Acad Sci U S A](#), 116 (2019) 4307-4315.

Cool Science

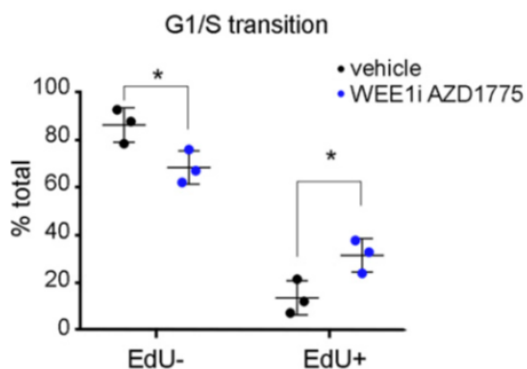
WEE1 Kinase Inhibitor AZD1775 Induces CDK1 Kinase-dependent Origin Firing in Unperturbed G1- and S-phase Cells

Mammalian cell division is controlled by specific proteins called cyclins that vary in expression through the different phases of the cell cycle. These proteins bind to and activate cyclin-dependent kinases (CDK) that phosphorylate key proteins to regulate entry and exit from specific cell cycle phases. WEE1 is a Ser/Thr kinase that is a key regulator of the G2/M transition. This exciting work by Dr. Chris Bakkenist and coworkers (Moiseeva et al., *Proc Natl Acad Sci U S A*, 116 (2019) 23891-23893.) investigated the action of the WEE1 kinase inhibitor AZD1775 (WEE1i) on the timing of origin firing in replicating cells. They showed that WEE1i induces CDK1-dependent RIF1 phosphorylation and CDK2- and CDC7-dependent activation of the replicative helicase, MCM2-7. WEE1 suppresses DK1 and CDK2 kinase activities to regulate the G1/S transition after the origin licensing is complete. They identified a role for WEE1 in cell cycle regulation and important effects of AZD1775, which is in clinical trials.

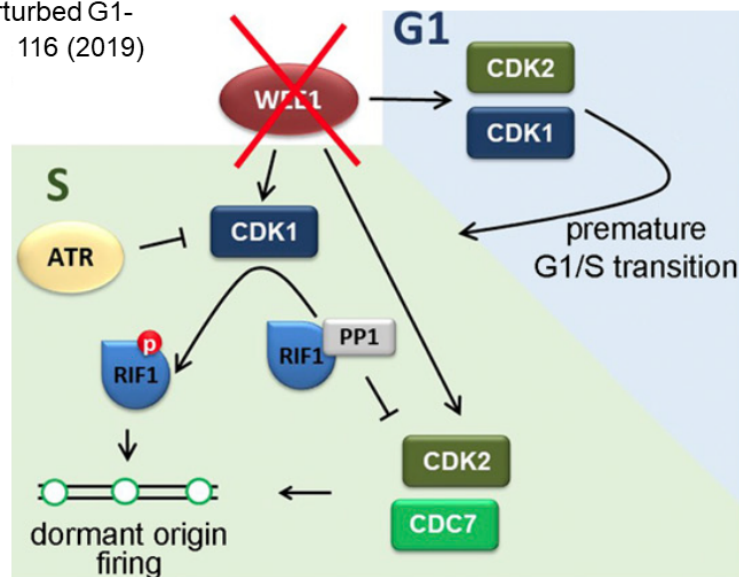
Impact: CDK1 drives the entire cell cycle with different mechanisms suppressing CDK1 activities in each stage: WEE1 in G1, WEE1/ATR in S, and ATR at the S/G2 transition. Accordingly, WEE1i and ATRi/CHK1i increase origin firing and this is associated with fork stalling and extensive regions of single-stranded DNA in cells that have yet to be treated with a DNA-damaging agent. These effects should be considered in the design and interpretation of clinical trials.

Funding: R01 CA204173 (C.J.B.), R00 CA207871 (H.U.O.), and P30CA047904.

T.N. Moiseeva, C. Qian, N. Sugitani, H.U. Osmanbeyoglu, C.J. Bakkenist, WEE1 kinase inhibitor AZD1775 induces CDK1 kinase-dependent origin firing in unperturbed G1- and S-phase cells, *Proc Natl Acad Sci U S A*, 116 (2019) 23891-23893.



Synchronized U2OS cells and treated G1/early S-phase cells with WEE1i. This treatment induced DNA synthesis in G1 and a premature G1/S transition.



Cartoon depicting the roles of WEE1 in controlling replication initiation.

More Cool Science

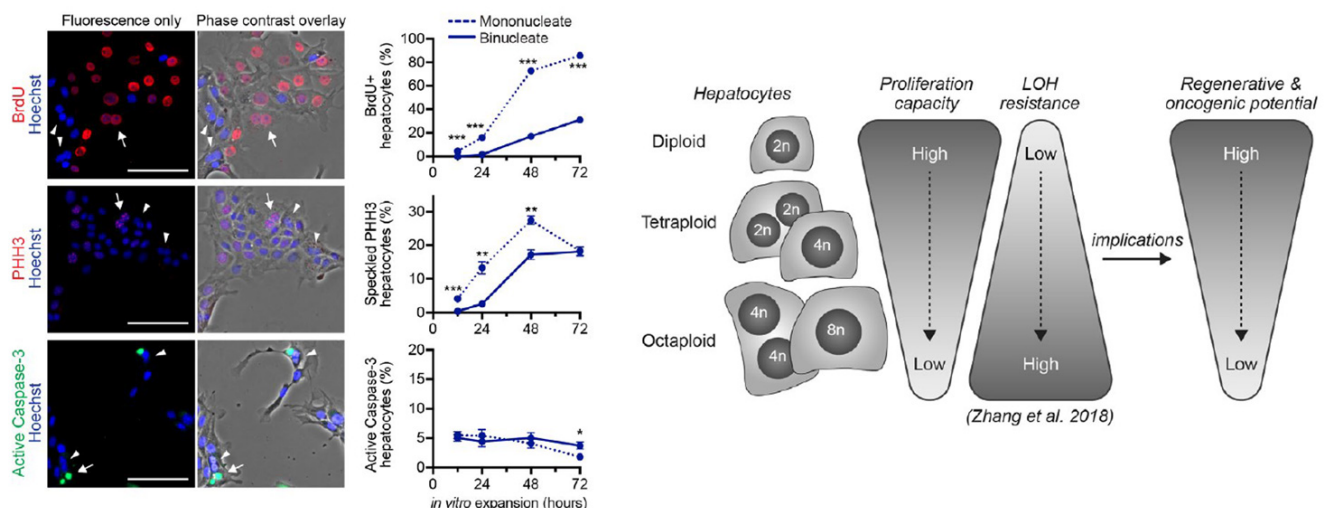
The Polyploid State Restricts Hepatocyte Proliferation and Liver Regeneration in Mice

The liver contains a mixture of hepatocytes with diploid or polyploid (tetraploid, octaploid, etc.) nuclear content. Polyploid hepatocytes are commonly found in adult mammals, representing ~90% of the entire hepatic pool in rodents. The cellular and molecular mechanisms that regulate polyploidization have been well characterized. However, it is unclear whether diploid and polyploid hepatocytes function similarly in multiple contexts. Answering this question has been challenging because proliferating hepatocytes can increase or decrease ploidy, and animal models with healthy diploid-only livers have not been available. Mice lacking E2f7 and E2f8 in the liver (liver-specific E2f7/E2f8 knockout; LKO) were recently reported to have a polyploidization defect, but were otherwise healthy. This present work by Andrew Duncan, PhD, and coworkers was able to rigorously characterize these LKO mice and demonstrated a 20-fold increase in diploid hepatocytes and maintenance of the diploid state even after extensive proliferation. (Wilkinson et al., *Hepatology*, 69 (2019) 1242-1258.) Livers from LKO mice maintained normal function but became highly tumorigenic when challenged with tumor-promoting stimuli, suggesting that tumors in LKO mice were driven, at least in part, by diploid hepatocytes capable of rapid proliferation. Indeed, hepatocytes from LKO mice proliferate faster and out-compete control hepatocytes, especially in competitive repopulation studies. In addition, diploid or polyploid hepatocytes from wild-type (WT) mice were examined to eliminate potentially confounding effects associated with E2f7/E2f8 deficiency. WT diploid cells also showed a proliferative advantage, entering and progressing through the cell cycle faster than polyploid cells, both in vitro and during liver regeneration (LR). Diploid and polyploid hepatocytes responded similarly to hepatic mitogens, indicating that proliferation kinetics are unrelated to differential response to growth stimuli.

Impact: Diploid hepatocytes proliferate faster than polyploids, suggesting that the polyploid state functions as a growth suppressor to restrict proliferation and tumor potential by the majority of hepatocytes.

Funding: R01 DK103645 (AWD) and the Commonwealth of Pennsylvania. E.C.S. was supported by an NIH/NIBIB training grant (T32-EB001026) entitled *Cellular Approaches to Tissue Engineering and Regeneration* and F31-DK112633.

P.D. Wilkinson, E.R. Delgado, F. Alencastro, M.P. Leek, N. Roy, M.P. Weirich, E.C. Stahl, P.A. Otero, M.I. Chen, W.K. Brown, A.W. Duncan, The Polyploid State Restricts Hepatocyte Proliferation and Liver Regeneration in Mice, *Hepatology*, 69 (2019) 1242-1258.



Functional differences between diploid and polyploid hepatocytes, including implications for liver regeneration (LR) and cancer. Diploid hepatocytes enter the cell cycle earlier and proliferate faster than polyploids, providing diploids with increased capacity for LR. In terms of liver cancer, diploids are uniquely sensitive to transformation because of tumor suppressor loss of heterozygosity and, when transformed, they are poised for rapid proliferation.

More Cool Science

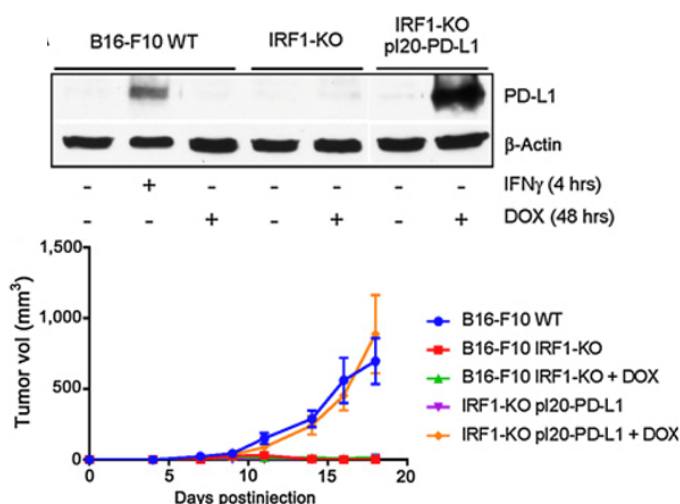
IRF1 Inhibits Antitumor Immunity Through the Upregulation of PD-L1 in the Tumor Cell

The interferon (IFN) regulatory factors (IRF) are transcription factors that are involved in cellular stress responses. Multiple studies have associated IRF1 with tumor-suppressive activities, partly by mediating immunosurveillance of tumors. In this highly collaborative study led by Dr. Saumendra Sarkar, working with the Bakkenist lab, this team uncovered an opposite tumor cell-intrinsic function of IRF1 in promoting tumor growth by using syngeneic mouse implantable tumor models. IRF1-deficient tumor cells showed reduced tumor growth in MC38 and CT26 colon carcinoma and B16 melanoma mouse models. This tumor growth reduction was dependent on host CD8⁺ T cells. No changes in the various T cell and myeloid cell populations were observed in the tumor infiltrating leukocytes. However, CD8⁺ T cells that had infiltrated IRF1-deficient tumors in vivo exhibited enhanced cytotoxicity. Interestingly, the IRF1-deficient tumor cells were unable to upregulate PD-L1 expression in vitro and in vivo and were more susceptible to killing by T-cells. Induced expression of PD-L1 in IRF1-deficient tumor cells restored tumor growth. These results showed IRF1 has a tumor-promoting activity in tumor cells, which is distinct from IRF1's role in the host immune cell.

Impact: Primary and acquired resistance to PD-1/PD-L targeted cancer therapy can arise from multiple factors. Results from this study indicate that IRF1 mediated upregulation of PD-L1 can cause reduced tumor cell killing and suggest that it may be possible to modulate the IRF1/IRF3 axis in tumor cells for targeted tumor therapy.

Funding: AI118896 (S.N.S.), CA178766 (S.N.S.), CA204173 (C.J.B.), P30CA047904 and R50CA211241.

L. Shao, W. Hou, N.E. Scharping, F.P. Vendetti, R. Srivastava, C.N. Roy, A.V. Menk, Y. Wang, J.-M. Chauvin, P. Karukonda, S.H. Thorne, V. Hornung, H.M. Zarour, C.J. Bakkenist, G.M. Delgoffe, S.N. Sarkar, IRF1 Inhibits Antitumor Immunity through the Upregulation of PD-L1 in the Tumor Cell, *Cancer Immunol Res*, 7 (2019) 1258-1266.



Induced PD-L1 expression (pl20-PD-L1 + Dox) rescues tumorigenicity of IRF1-KO mouse melanoma (B16-F10) cells in mice. Successful induction of PD-L1 induction with DOX, as shown by immunoblotting, in IRF1 knock out cells restores tumor growth to wild type levels.

More Cool Science

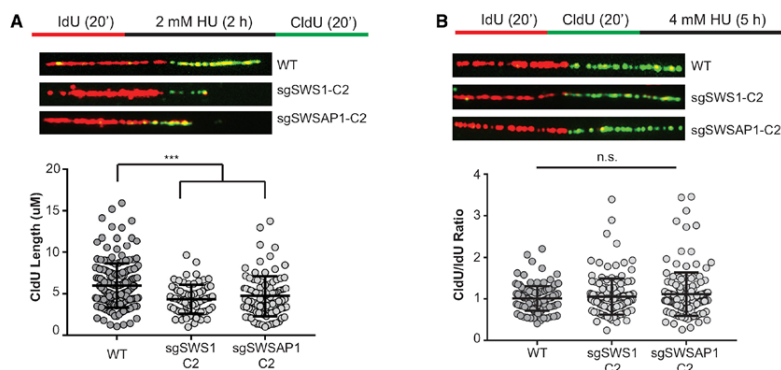
The Human Shu Complex Functions with PDS5B and SPIDR to Promote Homologous Recombination

RAD51 plays a central role in homologous recombination during double-strand break repair and in replication fork dynamics. RAD51 is regulated by many factors including the highly conserved Shu complex, and RAD51 misregulation is associated with genetic instability and cancer. In this collaborative study, led by Dr. Kara Bernstein, this team reported that the human Shu complex functions during DNA replication in regulating RAD51 recruitment to DNA repair sites, and during replication fork restart following replication fork stalling. Deletion of the Shu complex members SWS1 and SWSAP1 increased sensitivity to replication fork stalling and collapse caused by methyl methanesulfonate and mitomycin C exposure, caused a delayed and reduced RAD51 response, and caused fewer damage-induced sister chromatid exchanges. Additionally, this team identified SPIDR and PDS5B as novel Shu complex interacting partners that genetically function in the same pathway upon DNA damage. Collectively, this study uncovered a protein complex consisting of SWS1, SWSAP1, SPIDR and PDS5B, involved in DNA repair and provided new insight into Shu complex function and composition.

Impact: Deficiencies in RAD51 paralogs, such as RAD51C and RAD51D, are linked to familial breast and ovarian cancer predisposition, and homozygous mutation in SWS1 of the Shu complex has been associated with colorectal adenomatous polyposis. These findings highlight the importance of homologous recombination factors in the etiology of cancer. This study advances understanding of the highly conserved Shu complex, and provides evidence this complex is important for tolerance of DNA damage during replication by promoting RAD51-mediated functions.

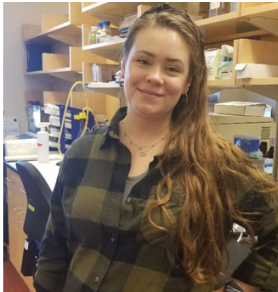
Funding: ES024872 (K.A.B.), CA207209 (R.J.O.), American Cancer Society Research Scholar Grants RSG-16-043-01-DMC (K.A.B.) and RSG-18-038-01-DMC (R.J.O.), and P30CA047904.

J. Martino, G.J. Brunette, J. Barroso-González, T.N. Moiseeva, C.M. Smith, C.J. Bakkenist, R.J. O'Sullivan, K.A. Bernstein, The human Shu complex functions with PDS5B and SPIDR to promote homologous recombination, *Nucleic Acids Res*, 47 (2019) 10151-10165

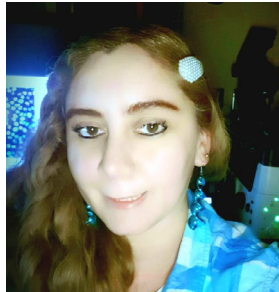


The Shu complex is needed to restart, but not protect, stalled replication forks. (A) Fork restart assay shows cells lacking Shu complex are deficient in fork restart (green CldU staining) after fork stalling with hydroxy urea. (B) Fork protection assay shows cells lacking Shu complex do not degrade forks (green CldU staining) after stalling with hydroxy urea.

Faculty and Staff News



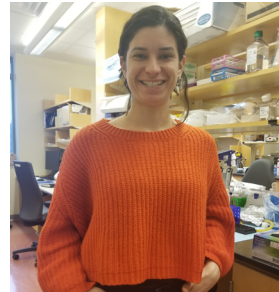
Megan Mahlke



Marlene Taja-Moreno



Pinakin Pandya



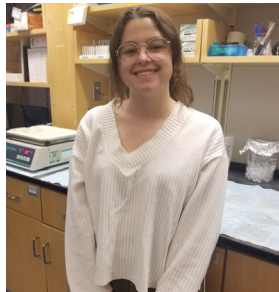
Lior Lumerman



Andrew Hefner



Shanna Ridgley



Rachel Jakielski

Congratulations, Lyblov Kublo and family on the arrival of their first grandchild, Lyina Kublo, born January 17, 2020, weighing 8 lbs. 8 oz.

Welcome back, **Vera Roginskaya** (Van Houten lab), recovering from shoulder/arm injury since late December 2019. We missed you!

Welcome new GSP students and staff members:

- **Marlene Taja-Moreno**, research specialist, **Lior Lumerman**, lab manager, and **Megan Mahlke, PhD**, postdoctoral associate, joined Dr. Yael Nechemia-Arbely's lab.
- **Pinakin Pandya, PhD**, postdoctoral associate, joined Dr. Jacob Stewart-Ornstein's lab.
- **Andrew Hefner**, lab technician, joined Dr. Heath Skinner's lab.
- **Shanna Ridgley**, student worker, joined the Genome Stability Program administrative team.
- **Rachel Jakielski**, undergraduate student researcher, joined the Van Houten lab.

Publications Interactome

