Fall 2020

DNA Pitt Crew

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

University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

Note from Director Robert L. Ferris, MD, PhD

The Genome Stability Program at UPMC Hillman Cancer Center reflects our focus on an important biological underpinning of cancer development and progression, with the overarching goals of gaining new insights into genome integrity and building on these insights to identify new ways to kill tumor cells.

Led by its phenomenal leaders Dr. Patricia Opresko and Dr. Ben Van Houten, the program has made crucial contributions to our understanding of DNA damage and repair, and many of these findings are being actively translated to the clinic. This year, UPMC Hillman Cancer Center achieved an exceptional score from the NCI on our CCSG grant, in no small part due to the scientific excellence of the Genome Stability Program. Furthermore, Dr. Opresko and Dr. Van Houten have consistently fostered a culture of mentorship and learning within the program, training the next generation of scientists at Hillman, who will no doubt go on to make important contributions in cancer research and other fields.

The accomplishments of our faculty, postdocs, and graduate students are a point of pride for Hillman, and I am pleased to support this opportunity for our community to read about them through the DNA Pitt Crew newsletter.

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

We are very pleased to present the Fall 2020 edition of the DNA Pitt Crew newsletter, which provides recent information about 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 incredibly challenging as we grappled with the lockdown in March and a gradual reopening of the labs in June under new safety precautions due to the global COVID-19 pandemic. Like much of the world, we have been adjusting to online meetings, scientific conferences, PhD thesis meetings and defenses, and for some members assisting children with online school. In addition, we are all coming to grips with recent events that sparked renewed awareness of the debilitating effects of systemic racism, and call to actively promote anti-racism and increase diversity and inclusion at our scientific institutions. We are incredibly proud of the accomplishments GSP members and Hillman collaborators have achieved during these difficult times—a true testament to their resilience and dedication. We are delighted to share highlights of recent impactful papers published in *Nature Chemical Biology, eLife, Nature Communications, and Science Advances*, among others. Trainees and new recruits had the opportunity to share their exciting projects at our weekly Work-in-Progress meetings and the annual GSP mini-retreat, held virtually. We were especially fortunate to have Dr. Simon Boulton deliver a spectacular keynote lecture to kick off our mini-retreat. Unfortunately, our in-person Pitt Stop visits from scientists were cancelled due to the pandemic, but many were rescheduled as virtual meetings. We wish everyone health and safety as we continue working during this challenging time and look forward to resuming our in-person meetings once the COVID-19 pandemic is contained.

Inside this issue:

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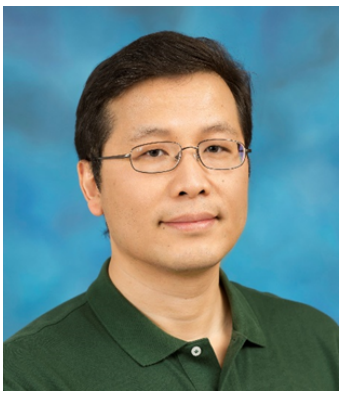
Ben Van Houten, PhD - vanhoutenb@upmc.edu



Dr. Lin Zhang

Faculty Spotlight: Lin Zhang, PhD

Dr. Lin Zhang is a professor 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. He received his bachelor's degree in Biochemistry from Sichuan University in China and his doctorate in Molecular Biology from the University of Southern California (USC). After postdoctoral training with Drs. Bert Vogelstein and Ken Kinzler at Johns Hopkins, he was recruited to join Hillman in 2002.

Dr. Zhang has tried to gain deep understanding of how cell death is initiated and executed in response to stress and drug treatment in colon cancer cells; how oncogenic driver mutations affect cell death signaling and ensuing antitumor immunity; why most of colorectal tumors respond poorly to therapeutic treatment; and what can be done to stimulate tumor cell killing and restore immunosurveillance of cancer. His long-term goal is to translate basic research findings on cell death to novel strategies and agents for improving colorectal cancer treatment and prevention. Dr. Zhang has authored over 130 research and review articles with an H-index of 57. His research has been continuously supported by the NCI since 2004, including two R01 grants started last year. He has served as a standing member of the NIH Drug Development and Molecular Pharmacology (DMP) study section and editorial boards of Cancer Research and other journals. He received honors such as V Scholar, General Motors Scholar (with a donated van still in the Hillman garage), American Cancer Society Research Scholar, and American Lung Association Career Investigator. At Hillman, he has served as chair of the Annual Retreat Committee and organized activities for the Colorectal Cancer Working Group. He is passionate about training and has mentored four PhD students and 15 postdoctoral fellows. He recently worked with Drs. Ben Van Houten and Patty Opresko to put together a Molecular Oncology T32 training grant which scored very well. In his spare time, he enjoys swimming, collecting stamps, and relaxing as a sports junkie by watching World Cup/ European soccer, Steelers and USC football, and NBA games.

Dr. Song My Hoang

Trainee Spotlight: Dr. Song My Hoang

Contributed by Ragini Bhargava, Nicole Kaminski, Michelle Lynskey, Roddy O'Sullivan

Dr. Song My Hoang is from Hanoi, Vietnam. She graduated from Union College, N.Y., with a degree in Biology and English. Dr. Hoang happened to minor in Irish Literature; thus, it seemed destiny would have her choose to join the O'Sullivan Lab, a place ideally suited to cultivate her shenanigans.

As a graduate student in the Molecular Pharmacology Graduate Student Program, she investigated the impact of poly ADP-ribose metabolism on telomere function in cancer cells deficient of ATRX chromatin remodeling complex. Through molecular and cellular studies, as well as an innovative proteomics system, Dr. Hoang characterized the role of the chromatin assembly factor, HIRA, in compensating for loss of ATRX in these cells. This study revealed HIRA as a potentially crucial factor that endows cancer cells with the ability to proliferate indefinitely. This work was presented in her doctoral dissertation, and her efforts led to her first authorship of a recent publication in *Nature Structural & Molecular Biology* and a review article in *Trends in Cancer.* She also contributed to a *Molecular Cell* paper from the lab in 2019.

Dr. Hoang earned many additional honors and awards over the years while pursuing her education including: ARDS RFBI Entrepreneurship Competition Semi-Finalist (2019); Pittsburgh Humanities Festival Speaker Finalist (2019); Departmental Honors (2015); Dean's List (2011-2015); Mayo Clinic Summer Research Fellow (2014); Davis Projects for Peace Finalist (2012); Council of International Schools, International Student Award (2011). In 2018-2019 she served as Biomedical Graduate Student Association Symposium Co-Chair for the University of Pittsburgh where she directed 20 graduate students, coordinated 25 faculty judges, six oral presentations and over 100 poster presentations.

Dr. Hoang has relocated to Boston where she accepted a position at Lumicks Capture Molecular Interaction as a field application scientist. She is already missed by her former colleagues in the O'Sullivan lab. Her playful pranks and sunny disposition meant there was never a dull moment in Hillman 2.7. We wish Dr. Hoang all the best in her future endeavors, as we know she will excel in all that she does.

Pitt Stop: Special Events and Visiting Speakers

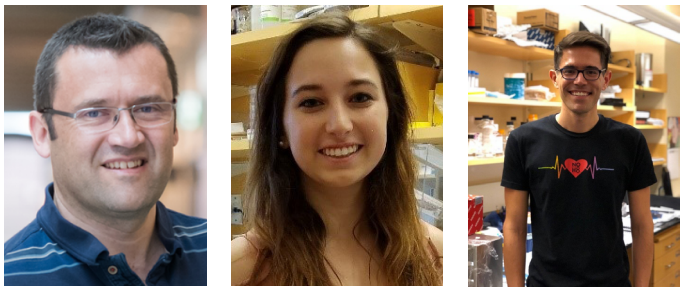
Due to COVID-19, our weekly GSP and Hillman Cancer Center Basic and Translational seminar series came to an unexpected interruption for a month while the University of Pittsburgh and Commonwealth of Pennsylvania went under strict stay-at-home orders. Work-in-Progress meetings resumed in May via Zoom.

Genome Stability Program Mini-Retreat: August 17, 2020

Contributed by Nicole Kaminski

The Genome Stability Mini-Retreat was held on August 17, 2020. The keynote speaker, Dr. Simon Boulton, tuned into the mini-retreat all the way from London, via Zoom, and delivered a riveting talk titled *Chromosome End Protection in Stem Cells and KSHV Roles in ALT.* Dr. Boulton specializes in double-stranded break repair metabolism and mechanisms. In addition to the keynote, some of the Hillman trainees presented their works in progress. Trainee talks presented were given by:

- **Nicole Kaminski** (O'Sullivan lab): *A novel role of HIRA in the alternative lengthening of telomeres pathway*
- **Samantha Sanford** (Opresko Lab): *Mechanisms of telomerase inhibition by therapeutic dNTPs*
- **Namrata Kumar** (Van Houten lab): *UV-damaged DNA binding protein (UV-DDB) stimulates the global genome repair of 8-oxoguanine through the recruitment of XPC and OGG1*
- **Braulio Bonilla** (Bernstein lab): *The yeast Shu complex promotes error-free tolerance of alkylation DNA damage*
- **Dayana Rivadeneira** (Delgoffe lab; collaborator in Cancer Immunology and Immunotherapy Program): *Metabolic pressures in the tumor microenvironment and their influence on T cell motility*
- **Shruthi Hamsanathan** (Gurkar lab): *DNA damage driven metabolic shift as a mediator of aging*
- **John Skoko** (Neumann lab): *Loss of peroxiredoxin1 function decreases homologous recombination through oxidation of a critical cysteine residue in RAD51*
- **Marilina Raices** (Yanowitz lab): *Characterization of the meiotic DNA double-strand break pathway*



Dr. Simon Boulton, Nicole Kaminski, and Dr. Ryan Barnes

Dr. Sylvie Doublie

Dr. Sylvie Doublie is a professor in the Department of Microbiology and Molecular Genetics at the University of Vermont and was featured at Hillman as our Basic & Translational Seminar Series speaker on October 20. Dr. Doublie's talk was titled *Structural analysis of mammalian DNA repair enzymes and cancer-associated mutations.*

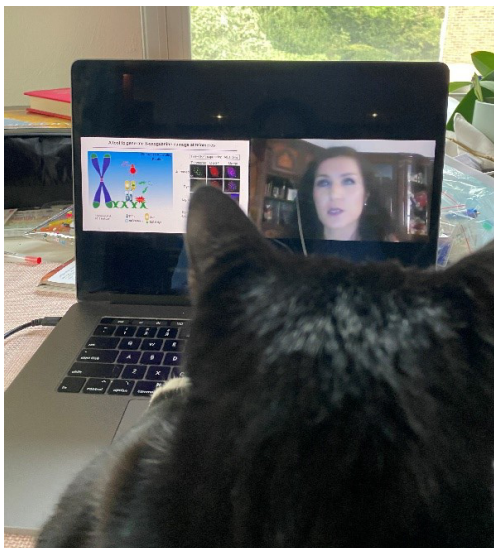
Scientific Conference Highlights and Awards

The Environmental Mutagenesis and Genomics Society held their 2020 Annual Meeting virtually

September 12-16, 2020

Several members of the Genome Stability Program attended the annual meeting of the Environmental Mutagenesis and Genomics Society, held for the first time virtually. The meeting was very well attended with over a thousand participants worldwide. GSP participation and presentations included:

- **Dr. Kara Bernstein** chaired a symposium on Environmental Genomics: Mechanisms & Approaches For Genomic Integrity Nucleosome Dynamics and Environmental Stress.
- **Dr. Patricia Opresko** chaired two DNA Repair Platforms with her former mentee and New Investigator Co-Chair, **Dr. Elise Fouquerel**, of Thomas Jefferson University; Dr. Opresko also served as a panelist in the EMGS Mentor/Mentee Panel "How do I get there? Career Panels to Guide Students, Postdocs, and New Investigators in Their Next Career Move" Sponsored by WEMGS and ESNIA.
- **Braulio Bonilla** (Bernstein lab) presented a seminar titled *The Yeast Shu Complex Functions in Error-Free Bypass of DNA Abasic Sites.*
- **Dr. Mariarosaria De Rosa** (Opresko lab) presented a seminar titled *Investigating the Role of Repair Enzyme MUTYH in Oxidative Damage to Telomeres*.
- **Dr. Ryan Barnes** (Opresko lab) presented a seminar titled *Telomeric 8oxoG Damage Induces Cellular Senescence by Disrupting Telomere Replication.*
- **Drs. Meghan Sullivan** and **Sarah Hengel** (Bernstein lab) and **Samantha Sanford** (Opresko lab) presented posters.



Pictured above is Dr. Mariarosaria De Rosa presenting from her home in Italy. Thanks to Samantha Sanford for the screenshot/photo.

Merrill J. Egorin Excellence in Scientific Leadership Award presented to Dr. Patricia Opresko

Contributed by Dr. Robert L. Ferris

I am honored and delighted to announce that Dr. Patty Opresko was selected as the 2020 recipient of "Merrill J. Egorin Excellence in Scientific Leadership Award." This award honors a faculty member that exemplifies scientific passion and scholastic dedication. Dr. Egorin focused his laboratory investigations on preclinical and clinical pharmacology studies designed to improve the development and use of anticancer agents. He was recognized nationally and internationally as a leader in the field of pharmacokinetics, and he was deeply committed to the academic success of our cancer center.

Dr. Opresko's skills as an exceptional scientist and outstanding mentor and her tireless leadership in cancer research are being recognized by this award. Among other things, we honor her work with the Genome Stability Program at UPMC Hillman Cancer Center. Her service and leadership on several key UPMC Hillman Cancer Center committees have played a pivotal role in facilitating Hillman's research mission. We believe that Dr. Opresko's contributions will allow UPMC Hillman Cancer Center to more effectively translate scientific discovery from the lab to the clinic to enhance patient care. In all of this, she embodies the principles of scientific excellence that were so characteristic of Dr. Egorin.

On behalf of the entire membership of UPMC Hillman Cancer Center and our Senior Leadership Team, I congratulate Dr. Opresko on being selected as this year's recipient of the Merrill J Egorin Excellence in Scientific Leadership Award. Dr. Opresko was honored at the Virtual UPMC Hillman Cancer Center Award recognition reception, which was held on October 13, 2020.

Dr. Patricia Opresko

Scientific Conference Highlights and Awards

Congratulations, Dr. Samantha Sanford

Contributed by Dr. Patty Opresko

Congratulations to Dr. Samantha Sanford (Opresko lab), on her Department of Infectious Diseases and Microbiology PhD Dissertation Defense and Final Examination, which took place on August 12, 2020. Dr. Sanford's work is focused on how modified nucleotides impact the ability of telomerase to lengthen telomeres, and has implications for cancer therapies and aging. Her dissertation title is *"Mechanisms of Telomerase Inhibition by Oxidized and Therapeutic dNTPs."* Dr. Sanford will continue her research as a postdoc in the Opresko lab for the time being.

Congratulations and Farewell, Dr. Darleny Lizardo

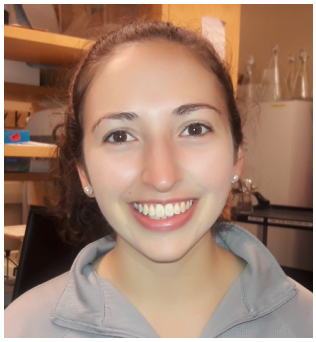
Contributed by Dr. Lin Zhang

Dr. Darleny Lizardo worked as a postdoctoral associate in the Zhang lab conducting research aimed at understanding the molecular mechanisms driving the interplay between cell death and immune response in microsatellite instability-high colorectal cancer. This year Darleny received a 2020 AACR Minority Scholar in Cancer Research Award. She 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. Ultimately, her goal is to lead a research laboratory at a small liberal arts college, to share her knowledge and give back. Darleny accepted a position as a scientist in bioanalytical science at Intellia Therapeutics, a biotech company in the Boston area. She will gain experience of working in industry, while trying to realize her dream of finding a teaching position at a university or college.

New Grant Awardees

Dr. Ryan Barnes (Opresko lab): New NIA grant titled ***Investigating the Impact of Telomere Specific Oxidative Base Damage in Cellular Aging***

Dr. Ben Van Houten: Two NIEHS grants titled ***Watching cooperative interactions between base and nucleotide excision repair proteins*** and ***Fourth EU-US Workshop on Nucleotide excision repair and crosslink repair-from molecules to mankind***



Congratulations to **Maria Beecher** and **Brittani Schable**, two graduate students in Dr. Van Houten's laboratory who have been awarded highly competitive T32 training slots. Maria is a third-year student in the Molecular Pharmacology Program working on UV-DDB stimulation of thymine DNA glycosylase, an important DNA repair enzyme involved in oxidative demethylation of 5-methylCytosine. Brittani is a second-year student in the Molecular Biophysics and Structural Biology Program working on single molecule studies of watching DNA repair proteins in real-time from purified proteins, through cell extracts and into living cells.

Hot Papers

1. Beckwitt EC, Jang S, Carnaval Detweiler I, Kuper J, Sauer F, Simon N, Bretzler J, Watkins SC, Carell T, Kisker C, Van Houten B. Single molecule analysis reveals monomeric XPA bends DNA and undergoes episodic linear diffusion during damage search. Nature communications. 2020;11(1):1356. Epub 2020/03/15. doi: 10.1038/s41467-020-15168-1. PubMed PMID: 32170071; PMCID: PMC7069974.

2. Guo QQ, Wang SS, Zhang SS, Xu HD, Li XM, Guan Y, Yi F, Zhou TT, Jiang B, Bai N, Ma MT, Wang Z, Feng YL, Guo WD, Wu X, Zhao GF, Fan GJ, Zhang SP, Wang CG, Cao LY, O'Rourke BP, Liu SH, Wang PY, Han S, Song XY, Cao L. ATM-CHK2-Beclin 1 axis promotes autophagy to maintain ROS homeostasis under oxidative stress. The EMBO journal. 2020;39(10):e103111. Epub 2020/03/19. doi: 10.15252/embj.2019103111. PubMed PMID: 32187724; PMCID: PMC7232007.

3. Hildreth AE, Ellison MA, Francette AM, Seraly JM, Lotka LM, Arndt KM. The nucleosome DNA entry-exit site is important for transcription termination and prevention of pervasive transcription. eLife. 2020;9. Epub 2020/08/28. doi: 10.7554/eLife.57757. PubMed PMID: 32845241; PMCID: PMC7449698.

4. Hoitsma NM, Whitaker AM, Beckwitt EC, Jang S, Agarwal PK, Van Houten B, Freudenthal BD. AP-endonuclease 1 sculpts DNA through an anchoring tyrosine residue on the DNA intercalating loop. Nucleic acids research. 2020;48(13):7345-55. Epub 2020/06/17. doi: 10.1093/nar/gkaa496. PubMed PMID: 32542366; PMCID: PMC7367167.

5. Kapralov AA, Yang Q, Dar HH, Tyurina YY, Anthonymuthu TS, Kim R, St Croix CM, Mikulska-Ruminska K, Liu B, Shrivastava IH, Tyurin VA, Ting HC, Wu YL, Gao Y, Shurin GV, Artyukhova MA, Ponomareva LA, Timashev PS, Domingues RM, Stoyanovsky DA, Greenberger JS, Mallampalli RK, Bahar I, Gabrilovich DI, Bayır H, Kagan VE. Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. Nature chemical biology. 2020;16(3):278-90. Epub 2020/02/23. doi: 10.1038/s41589-019-0462-8. PubMed PMID: 32080625; PMCID: PMC7233108.

6. Rodríguez A, Zhang K, Färkkilä A, Filiatrault J, Yang C, Velázquez M, Furutani E, Goldman DC, García de Teresa B, Garza-Mayén G, McQueen K, Sambel LA, Molina B, Torres L, González M, Vadillo E, Pelayo R, Fleming WH, Grompe M, Shimamura A, Hautaniemi S, Greenberger J, Frías S, Parmar K, D'Andrea AD. MYC Promotes Bone Marrow Stem Cell Dysfunction in Fanconi Anemia. Cell stem cell. 2020. Epub 2020/10/01. doi: 10.1016/j.stem.2020.09.004. PubMed PMID: 32997960.

7. Schaich MA, Sanford SL, Welfer GA, Johnson SA, Khoang TH, Opresko PL, Freudenthal BD. Mechanisms of nucleotide selection by telomerase. eLife. 2020;9. Epub 2020/06/06. doi: 10.7554/eLife.55438. PubMed PMID: 32501800; PMCID: PMC7274783.

8. Wang W, Douglas D, Zhang J, Kumari S, Enuameh MS, Dai Y, Wallace CT, Watkins SC, Shu W, Xing J. Live-cell imaging and analysis reveal cell phenotypic transition dynamics inherently missing in snapshot data. Science advances. 2020;6(36). Epub 2020/09/13. doi: 10.1126/sciadv.aba9319. PubMed PMID: 32917609; PMCID: PMC7473671. Epithelial to mesenchymal transition

9. Xie W, Jiao B, Bai Q, Ilin VA, Sun M, Burton CE, Kolodieznyi D, Calderon MJ, Stolz DB, Opresko PL, St Croix CM, Watkins S, Van Houten B, Bruchez MP, Burton EA. Chemoptogenetic ablation of neuronal mitochondria in vivo with spatiotemporal precision and controllable severity. eLife. 2020;9. Epub 2020/03/18. doi: 10.7554/eLife.51845. PubMed PMID: 32180546; PMCID: PMC7077989.

10. Xu J, Ma H, Ma H, Jiang W, Mela CA, Duan M, Zhao S, Gao C, Hahm ER, Lardo SM, Troy K, Sun M, Pai R, Stolz DB, Zhang L, Singh S, Brand RE, Hartman DJ, Hu J, Hainer SJ, Liu Y. Super-resolution imaging reveals the evolution of higher-order chromatin folding in early carcinogenesis. Nature communications. 2020;11(1):1899. Epub 2020/04/22. doi: 10.1038/s41467-020-15718-7. PubMed PMID: 32313005; PMCID: PMC7171144.

Cool Science

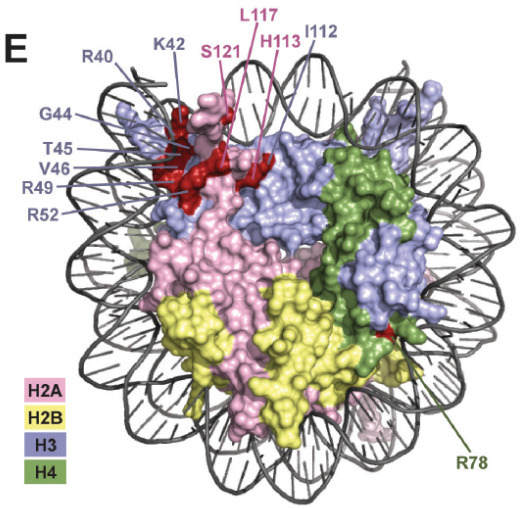
The Nucleosome DNA Entry-Exit Site is Important for Transcription Termination and Prevention of Pervasive Transcription.

The role of chromatin in transcription termination is poorly understood, and defects in this process increase aberrant readthrough transcripts which are observed in numerous cancers. To address the role of nucleosomes in transcription termination, Dr. Arndt and colleagues performed an unbiased genetic screen in yeast to identify histone mutants defective in this process. They discovered a novel function for the nucleosome DNA entry-exit site, a region that normally controls nucleosome stability and specific histone modifications, in preventing transcription terminator readthrough and in controlling noncoding transcription. These mutants also reduced nucleosome occupancy. When they restored nucleosome occupancy with a nucleosome superbinder sequence, they discovered the DNA entry-exit site regulates termination by controlling nucleosome stability and imposing a barrier to RNA polymerase II progression. Collectively, their studies suggest that nucleosomes can facilitate transcription termination by acting as a barrier to transcription, and highlight the importance of the nucleosome DNA entry-exit site in broadly maintaining the integrity of the transcriptome.

Impact: This study opened new lines of investigation identifying the mechanisms by which regulatory proteins target the nucleosome DNA entry-exit site to modulate its role in controlling genome access and preventing accumulation of aberrant transcripts observed in cancer.

Funding: R01GM052593 to KMA, F31GM129917 to MAE, Andrew Mellon and Margaret A Oweida predoctoral fellowships to AEH, a K Leroy Irvis Fellowship to AMF and a Colella Research Fellowship to LML.

Hildreth AE, Ellison MA, Francette AM, Seraly JM, Lotka LM, Arndt KM. The nucleosome DNA entry-exit site is important for transcription termination and prevention of pervasive transcription. eLife. 2020;9. Epub 2020/08/28. PubMed PMID: 32845241; PMCID: PMC7449698.



X-ray crystal structure of the nucleosome showing histone residues (red) identified in the termination reporter screen. H2A, H2B, H3, and H4 are colored in pink, yellow, lilac, and green, respectively. Structure from PDB ID 1ID3

Redox Lipid Reprogramming Commands Susceptibility of Macrophages and Microglia to Ferroptotic Death.

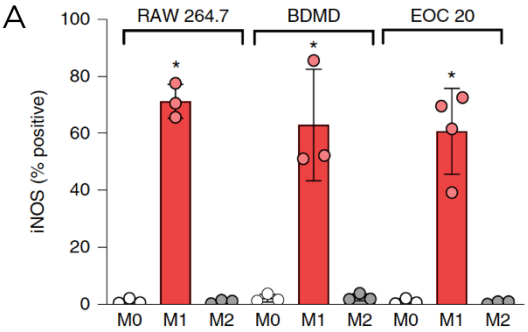
Ferroptosis is a type of redox-driven cell death that is common in pro-inflammatory and pro-oxidant conditions, such as the tumor microenvironment. In an inter-programmatic collaboration, this team discovered that activated M1 macrophages and microglia are resistant to ferroptosis due to strong upregulation of inducible nitric oxide synthetase (iNOS), which produces nitric oxide (NO). Conversely, activated M2 macrophages and microglia do not upregulate iNOS and are highly sensitive to ferroptosis, but are protected upon addition of exogenous NO donors. This multidisciplinary team, involving inter-programmatic collaborations, led by Dr. Valarian Kagan, proposes that the anti-ferroptotic effect of NO is likely due to its ability to react with lipid metabolizing enzymes that generate pro-ferroptotic signals. Overall, this study provides novel evidence that iNOS is a potent regulator of ferroptotic cell death.

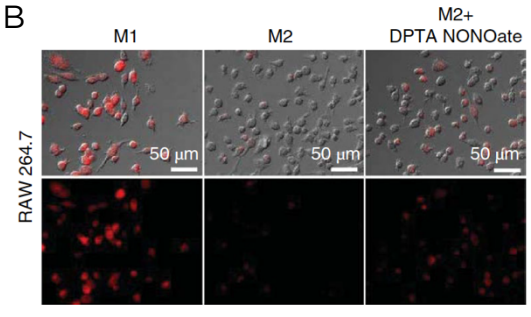
Impact: This study demonstrates a specific role for macrophage derived nitric oxide in protecting cells from ferroptotic cell death and has important implications for macrophage and microglia survival in the tumor microenvironment.

Funding: HL114453-06, U19AI068021, CA165065-06, NS076511, NS061817, P41GM103712 and by Russian academic excellence project '5-100'.

Funding: K99ES027028 to EF; R21ES025606 to PLO, SCW and MPB; R01CA207342, R01ES028242 and R01ES022944 to PLO; R01EB017268 to MPB; P30CA047904 to UPMC Hillman Cancer Center.

Kapralov AA, Yang Q, Dar HH, Tyurina YY, Anthonymuthu TS, Kim R, St Croix CM, Mikulska-Ruminska K, Liu B, Shrivastava IH, Tyurin VA, Ting HC, Wu YL, Gao Y, Shurin GV, Artyukhova MA, Ponomareva LA, Timashev PS, Domingues RM, Stoyanovsky DA, Greenberger JS, Mallampalli RK, Bahar I, Gabrilovich DI, Bayır H, Kagan VE. Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. Nature chemical biology. 2020;16(3):278-90. Epub 2020/02/23. PubMed PMID: 32080625; PMCID: PMC7233108.

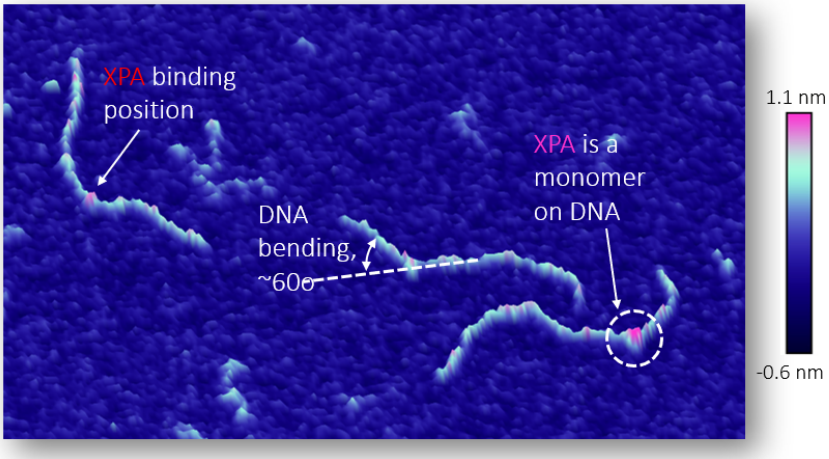


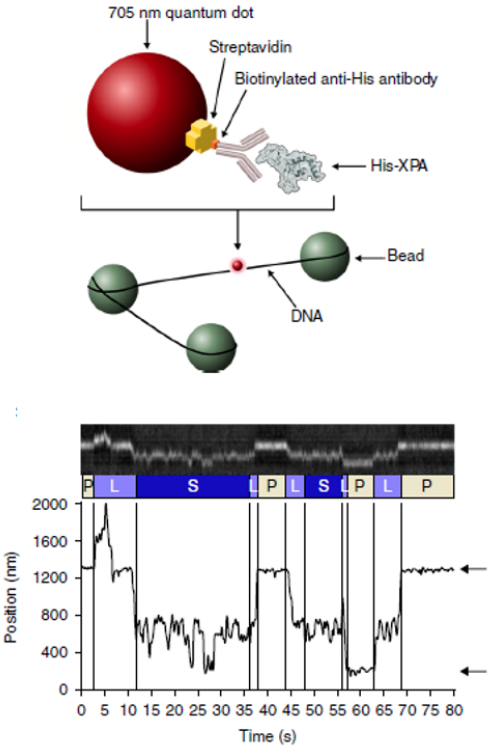


Activated M1, but not M2, macrophages (a) overexpress inducible nitric oxide synthetase (iNOS) and (b) produce more nitric oxide (NO) as indicated with a fluorescent NO probe and NONOate as a positive control.

More Cool Science

AFM image of a 538 bp piece of DNA containing a lesion 30% from one end, XPA is pink, and DNA is light blue





Quantum dot labeled XPA on a DNA tightrope (top). XPA paused linear motion over time is shown (bottom). Arrows indicate two pause sites (P); L = long range motion > 690 nm, and S = short range motion 130-690 nm.

Single Molecule Analysis Reveals Monomeric XPA Bends DNA and Undergoes Episodic Linear Diffusion During Damage Search.

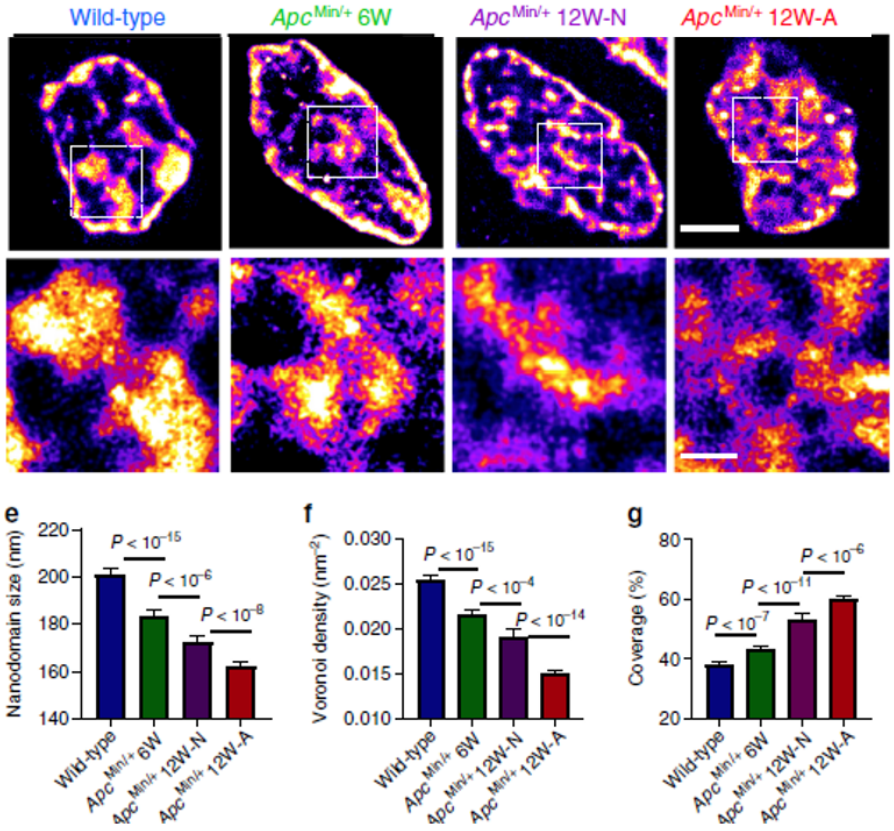
When we go out in the sun our exposed skin gets damaged by ultraviolet light producing specific types of lesions in the DNA, which are removed through a process of nucleotide excision repair (NER), orchestrated by some 30 proteins. Defects in the function of at least seven of these proteins in humans causes a disorder called xeroderma pigmentosum characterized by sun sensitivity and a 2,000 fold increase in skin cancer and in some cases neurodegeneration. One such protein XPA plays a central role in this process. In order to understand how XPA interacts with DNA to help perform NER, in this international collaboration, Dr. Emily Beckwitt (working in Dr. Bennett Van Houten's laboratory) used two single-molecule techniques to investigate how XPA discriminates DNA lesions from non-damaged DNA. Atomic force microscopy showed that XPA has high specificity for damage, and binds as a monomer at sites of damage causing a site-specific bend of 60°. Working with Dr. Simon Watkins at the Center for Biologic Imaging, Dr. Beckwitt watched single molecules of fluorescently labeled XPA scan DNA for damage sites and found that the protein underwent specific pauses at discrete sites following by rapid linear diffusion to another site.

Impact: This highly innovative work captures the movement of XPA on DNA as it searches for DNA lesions. XPA is believed to fold and unfold as it encounters damage sites allowing interaction with other repair proteins resulting in paused motion on DNA. It is believed that this limited linear diffusion along DNA helps relatively low abundance repair proteins scan a sea of non-damage DNA for sites of damage in the human nucleus.

Funding: R01ES019566 & R01ES028686 to B.V.H., T32GM088119 to E.C.B., and 2P30CA047904 to UPMC Hillman Cancer Center; Deutsche Forschungsgemeinschaft, SFB-1361, TP2-Carell.

Beckwitt EC, Jang S, Carnaval Detweiler I, Kuper J, Sauer F, Simon N, Bretzler J, Watkins SC, Carell T, Kisker C, Van Houten B. Nature Communications. 2020;11(1):1356.

More Cool Science



Super-resolution imaging of DAPI-stained DNA in Apc$^{Min/+}$ mouse model.

STORM images of individual nuclei showing DNA folding

Panel e: size of the nanodomains of DNA
Panel f: local density of the DNA
Panel g: percentage coverage of occupied DNA domains.

Note that with tumor formation and progression tumors show decrease nanodomain size, decreased density, but increase nuclear coverage, indicating DNA unfolding and a large chromatin reorganization in the tumor tissue.

Super-resolution Imaging Reveals the Evolution of Higher-order Chromatin Folding in Early Carcinogenesis.

Genomic DNA is folded into a higher-order structure that regulates transcription and maintains genomic stability. Although progress has been made on understanding biochemical characteristics of epigenetic modifications in cancer, the in-situ higher-order folding of chromatin structure during malignant transformation remains largely unknown. In this multi-investigator study, using optimized stochastic optical reconstruction microscopy (STORM) for pathological tissue (PathSTORM), a group led by Dr. Yang Liu uncovered a gradual decompaction and fragmentation of higher-order chromatin folding throughout all stages of carcinogenesis in multiple tumor types, and prior to tumor formation. Their integrated imaging, genomic, and transcriptomic analyses reveal functional consequences in enhanced transcription activities and impaired genomic stability.

Impact: This study demonstrates the potential of imaging higher-order chromatin disruption to detect high-risk precursors that cannot be distinguished by conventional pathology. Taken together, their findings reveal gradual decompaction and fragmentation of higher-order chromatin structure as an enabling characteristic in early carcinogenesis to facilitate malignant transformation, which may improve cancer diagnosis, risk stratification, and prevention.

Funding: R33CA225494, R01CA185363 (to Y.L.), R35GM133732 (to S.J.H.), R01CA101753-14 (to S.S.), P30CA047904 and Henry L. Hillman Foundation (to R.E.B.).

Xu J, Ma H, Ma H, Jiang W, Mela CA, Duan M, Zhao S, Gao C, Hahm ER, Lardo SM, Troy K, Sun M, Pai R, **Stolz DB, Zhang L, Singh S,** Brand RE, Hartman DJ, Hu J, Hainer SJ, Liu Y.N Nature Communications. 2020;11(1):1899. Bold = HCC program members.

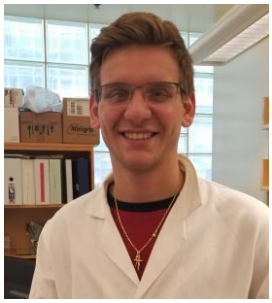
Faculty and Staff News

We're pleased to welcome (and in some cases, welcome back) the following new staff members:

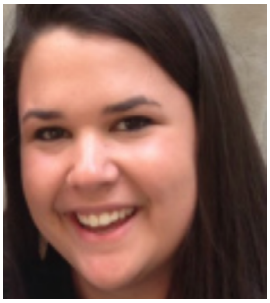
- **Andrew Hefner**, research technician and **Phillip Pifer, MD, PhD**, Radiation Oncology resident, joined Dr. Heath Skinner's laboratory.
- **Andrew Cipriano**, research specialist, joined the Bakkenist laboratory.
- **Anna Wondisford**, graduate medical student, joined the O'Sullivan laboratory.
- **Brittani Schnable** (received a slot on the Molecular Biophysics and Structural Biology T32 Training Grant) and **Sripriya "Priya" Raja**, graduate students in Pharmacology and Chemical Biology, joined the Van Houten laboratory.
- **James "Trey" Harkness,** graduate student in the Molecular Pharmacology Graduate Program, rotated to the Opresko laboratory. Trey is in the Interdisciplinary Biomedical Graduate Program doing a research rotation in Opresko laboratory this semester.
- **Taylor Gatesman**, new Interdisciplinary Biomedical Graduate Program doing a research rotation in the Bakkenist laboratory.
- **Sophia Grace Cosentino**, BSBA, joined as the new administrator in Hillman Cancer Center Research Pavilion, Suite 2.6. Sophia provides administrative support for Drs. Chris Bakkenist, Ravi Patel, Heath Skinner, Shikhar Uttam, and their labs. Sophia is a graduate of the University of Pittsburgh class of 2020. She was a student worker in the Department of Biomedical Engineering where she compiled the department's annual report. Despite her young age, she brings a lot of Pitt experience. Sophia graduated from PPS CAPA High school at age 16 and college at 20. In addition to her many responsibilities at Hillman Cancer Center, Sophia serves as worship leader at the Bellefield Presbyterian church in Oakland, where her mother serves as administrator. Of note, her father is a musician in the famous Pittsburgh Boilermakers Jazz band.
- **Natalie King, BA**, joined the Genome Stability Program as our administrator supporting Drs. Patty Opresko, Roddy O'Sullivan and Ben Van Houten. Natalie comes from Pitt's Department of Psychology where she served as business coordinator for two years. Natalie obtained her bachelor's degree in liberal arts form Penn State University.

Andrew Hefner

Phillip Pifer



Andrew Cipriano



Anna Wondisford

Brittani Schnable

Priya Raja

Trey Harkness

Taylor Gatesman

Sophia Cosentino

Natalie King

More Faculty and Staff News

Pictured above Laura Garcia Exposito and Jonathan Barroso-Gonzalez, former post-docs in Dr. Roddy O'Sullivan lab. Jonathan returned to Spain to reunite with Laura and their beautiful family. Laura finished her post-doctoral studies and returned home to Spain last June 2019 with their children

Congratulations and best wishes to Dr. Jonathan Barroso Gonzalez, former postdoctoral associate of Pharmacology and Chemical Biology. Dr. Barroso Gonzalez completed his second postdoc at Pitt in June 2020 in the O'Sullivan lab with focus on the characterization of RAD51AP1 in the Alternative Lengthening of Telomeres (ALT) pathway. Having made some surprising discoveries, Jonathan's work was published in Molecular Cell in 2019. Following this, Jonathan proceeded to examine other critical regulators of ALT, the results of which will be forthcoming. He returned to his home of Tenerife in the Canary Islands in June 2020. Basking in the sunshine and waves of his island paradise, Jonathan has taken up wine making, as he awaits news of further research positions in Spain. Jonathan is very much missed in the O'Sullivan lab, where his colleagues know that the future is bright for this "renaissance man."

Barroso-Gonzalez J., García-Exposito L., Hoang SM., Lynskey ML., Roncaioli JL., Ghosh A., Wallace CT., Modesti M., Bernstein KA., Sarkar SN., Watkins SC., O'Sullivan RJ. "RAD51AP1 Is an Essential Mediator of Alternative Lengthening of Telomeres". Molecular Cell, 2019, 76(1):11-26. IF 14.548.

*Article recommended on the Faculty1000 website (Karlseder J: F1000Prime Recommendation of [Barroso-González J et al., Mol Cell 2019 76(1):11-26.e7]. In F1000Prime, 13 Sep 2019; 10.3410/f.736399248.793564861).

Shanna Ridgley

Congratulations, best wishes, and thanks to Katie Lemon, research technician in Dr. Chris Bakkenist's lab. Katie recently accepted a research coordinator position at UPMC Children's Hospital of Pittsburgh in the Ophthalmology Department. Katie's smile, joyfulness, and kindness will be missed by all those in the 2.1, 2.6, and 2.7 Hillman Cancer Center labs. Katie also works part-time as an emergency room technician at St. Margaret's Hospital and at a local YMCA. In addition to working several jobs, she volunteers at a local homeless shelter.

Farewell and best wishes to Marlene Taja Moreno of Dr. Yael Arbely's lab, where she worked as a research specialist. Marlene decided to go home to Mexico to pursue her PhD studies close to family. We wish Marlene all the best in her future endeavors.

Shayla Goller

Thank you and best wishes to Shanna Ridgley, GSP undergraduate student worker, University of Pittsburgh, who graduated with her BS in April. Her time was cut short due to COVID-19, but she helped with the last GSP newsletter and scheduling WIP conferences during the five to six weeks she worked for the Genome Stability Program. Shanna plans to be a dentist, and currently is in her first year of studies at the University of Pittsburgh School of Dental Medicine.

Good luck, Shayla Goller, research technician from Dr. Jacob Stewart-Ornstein's lab, who has moved on to new a position at UPMC Children's Hospital of Pittsburgh working with the COVID-19 vaccine.

From Mary Byrnes

Goodbye and best wishes to all on my last day at the University of Pittsburgh, September 30, 2020, due to Pitt Staff Early Retirement Program. I believe that cancer does not have a fighting chance with you all in the ring! Wishing you all the best in your future endeavors.

Mary Byrnes