June 2022

DNA Pitt Crew

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

University of Pittsburgh

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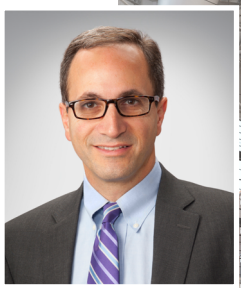
Note from Director Robert L. Ferris, MD, PhD



One of the important aspects of my job is to expand our ability to develop novel approaches to treat patients with cancer. I am delighted to report that during the next few months, the UPMC Hillman Cancer Center is undergoing a significant expansion into the Assembly building directly beside the Research Pavilion, which we have occupied for almost 20 years. The Assembly is a beautifully renovated building in the former Ford Motor Company Assembly Plant built in 1915 and is on the National Register of historic places. With state-of-the-art laboratories and facilities, the Assembly is expected to house the Women's Cancer Research Center (with Magee-Womens Research Institute), the Tumor Microenvironment Center, Precision Oncology (with the Institute for Precision Medicine), Cancer & Aging (with the Aging Institute), and the Center for Radiological Translation. Over 50 UPMC Hillman investigators will occupy the wet and dry labs in the building, which will provide a synergistic environment to inspire collaborative research that can lead to novel therapies for treating patients with cancer. This goal is at the heart of what we do at UPMC Hillman Cancer Center, and, as you will see from this spring's DNA Pitt Crew newsletter, a goal that the Genome Stability Program (GSP) contributes to significantly.

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

We are delighted to present the Spring 2022 edition of the DNA Pitt Crew newsletter, which provides recent information about UPMC Hillman Cancer Center (HCC) Genome Stability Program (GSP) members, new recruits, and some of the exciting scientific accomplishments from this program. With the slow return to travel, many of us enjoyed our first in-person scientific meeting since the pandemic started. The GSP was particularly well represented at the recent Gordon Research Conference and Seminar on Mammalian DNA Repair with talks by faculty, postdocs and students. This edition includes four scientific highlights of recently published impactful studies that: 1) show how MMR protein MutSα retrains the alternative lengthening of telomeres pathway in cancer cells *(Cell Reports)*; 2) define how CDK4/6 inhibitors sensitize cancer cells to chemotherapy and immune checkpoint blockade *(Cancer Research)*; 3) demonstrate how two major DNA repair pathways converge to mediate removal of the common lesion 8-oxoguanine *(Nature Communications)*; and 4) develop a robust imaging platform for chromatin structures and applications for stratifying cancer patients *(Sci Advances)*. We also highlight a new translational study from Dr. Heath Skinner's lab *(Nature Communications)* and an exciting intra-programmatic publication from junior faculty, Dr. Aditi Gurkar's lab in collaboration with senior investigators, Drs. Bayir and Kagan. We are pleased to share new grants and awards to GSP members and are especially proud of the K99 postdoctoral fellow awardees. We look forward to our annual GSP retreat to be held in-person this year on June 21st in the new Assembly building. We wish everyone good health and safety as we continue working during this challenging time and look forward to a return to in-person meetings.

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Judith Yanowitz

Faculty Spotlight: Judith Yanowitz

Judith Yanowitz, PhD, is an associate professor of obstetrics, gynecology, and reproductive sciences at the University of Pittsburgh (Pitt) and a member of the UPMC Hillman Cancer Center and the Magee-Womens Research Institute (MWRI). Her laboratory uses the nematode *Caenorhabditis elegans* to interrogate how genome integrity is faithfully maintained across generations.

Dr. Yanowitz became enamored with the idea of pursuing a career in biology when her father gave her a Scientific American article on the neuromuscular junction and introduced her as a young teen to the burgeoning field of genetic engineering. However, it was not until after college, when working as a technician at Rockefeller University in Dr. Titia de Lange's laboratory, that she fell in love with chromosomes and committed herself to pursuing an academic career. She studied Drosophila sex determination and dosage compensation for her PhD studies in Dr. Paul Schedl's laboratory at Princeton University before joining Dr. Andrew Fire's laboratory at the Carnegie Institution for Science in Baltimore where she first trained in C. elegans developmental biology. This led her to attain a Staff Associate position at Carnegie, where she began her independent research program, investigating the factors that regulate meiotic double-strand break formation and repair. She joined MWRI and Pitt in November 2009.

The research in the Yanowitz laboratory focuses on revealing conserved mechanisms that maintain genome integrity with the hope that it will inform understanding of the dysfunction that leads to cancer, aging and infertility. Her work on various aspect of germ cell development have led to seminal papers in *Nature*, *Development*, and *Current Biology*, among other publications. Of note, her laboratory identified a chromosome- intrinsic, meiotic surveillance system that ensures that each chromosome receives a crossover. Her laboratory is currently funded by multiple NIH grants to understand the regulation of this checkpoint, to investigate the role(s) of the proteasome in meiotic break formation and repair, and to interrogate the function of germ cell nuclear antigen (GCNA) in the repair of DNA:protein crosslinks in germ cells.

In addition to her research efforts, Dr. Yanowitz is the MWRI Confocal Core Director and the MWRI Director of Research Education. In the latter role, she oversees MWRI's STEM programs, promotes training of graduate students and post-doctoral fellows, and is developing a program for high school teachers. She is an active member of the Integrative Systems Biology and Interdisciplinary Biomedical graduate programs and is a faculty mentor in the "Springboard" program for new faculty investigators. She has two daughters with her husband, Dr. Harry Hochheiser, who is an associate professor in biomedical informatics, also at Pitt. Dr. Yanowitz usually bikes to work and in her free time, stays active, enjoys reading (mostly fiction), and dabbles in photography.

Ryan Barnes

Trainee Spotlight: Ryan Barnes

Congratulations to Ryan Barnes, PhD, a postdoctoral fellow in Dr. Patricia Opresko's lab, on his newly awarded K99/R00 grant from the National Institute of Environmental Health Sciences (NIEHS). Dr. Barnes earned his PhD at the Pennsylvania State University College of Medicine in 2017 in the lab of Dr. Kristin Eckert, where he studied DNA polymerases during replication stress and the replication of common fragile site sequences. Soon after, Dr. Barnes joined the Opresko lab at the UPMC Hillman Cancer Center, where he utilized his biochemical expertise to develop an assay to demonstrate the lab's new tool (FAP-TRF1) specifically induces 8-oxo-guanine at telomeres. This and other contributions resulted in a second authorship on the important 2019 Molecular Cell manuscript from the lab demonstrating that targeted and persistent oxidative base damage accelerates telomere shortening and loss in human cancer cells.

Utilizing the FAP-TRF1 tool, Dr. Barnes developed non-diseased cell models to study the impact of oxidative base damage at telomeres on cell aging. He discovered that surprisingly, and unlike in cancer cells, a single induction of telomere base oxidation is sufficient to promote cellular senescence, in the absence of telomere shortening. Instead, Dr. Barnes discovered senescence induction was triggered by telomere fragility caused by replication stress and was dependent on both cell replication and p53. This work is available as a preprint in bioRxiv and recently accepted for publication in Nature Structural & Molecular Biology. In addition to his major research, Ryan has collaborated on several projects within the Opresko lab, and with other labs around the country resulting in three co-author publications and two that are in preparation.

In addition to his K99/R00, Dr. Barnes has received numerous awards as a postdoctoral fellow including a Hillman Postdoctoral Fellowship for Innovative Cancer Research and an F32 from the National Institute on Aging. He was awarded an Environmental Mutagenesis and Genomics Society (EMGS) travel award in 2018 and 2019, and 2nd place for best platform presentation at the EMGS 2019 annual meeting. He was elected co-chair of the 2020 Gordon Research Conference (GRC) on DNA Damage, Mutation and Cancer. His abstract was selected for an oral presentation at numerous conferences including the GRS on DNA Damage, Mutation and Cancer (2022), GRC on Mammalian DNA Repair (2021), EMGS Meeting (2021, 2020, 2019, 2018), and the CSHL Meeting on Telomeres and Telomerase (2021, 2019).

Dr. Barnes is continuing his current work on oxidative base damage induced replication stress at telomeres in the Opresko lab and using CRISPR/Cas9 screening technology to uncover novel factors regulating this. With his newly funded K99/R00 award, Dr. Barnes plans to continue his research in oxidative stress using the FAP technology and studying the environmentally relevant UVA exposures when he establishes his own independent lab. He plans to begin applying for faculty positions this spring and summer.

Dr. Opresko says "Dr. Barnes is the complete package and has demonstrated excellence in all aspects of academic science including research, leadership, communication, teaching and innovation. He is a highly skilled experimentalist and creative thinker and has been a joy to work with". We are so excited for Dr. Barnes's future and wish him the best of luck as he applies for faculty positions and transitions to an independent research program.

Sarah Hengel

Trainee Spotlight: Sarah Hengel

Sarah Hengel, PhD, is from the Midwest and graduated Cum Laude from the College of St. Scholastica in Duluth, MN with a B.S. in Biochemistry. As an undergrad, they learned about the fascinating world of protein structure/function by measuring the motions of a PDZ domain protein using a structural biology technique called Nuclear Magnetic Resonance. Dr. Hengel then went on to study with single-molecule protein biophysicist, Dr. Maria Spies, in the Department of Biochemistry at the University of Iowa. There, Dr. Hengel used biochemistry and biophysics to identify lead compounds for BRCA1/2 breast cancers by targeting the DNA repair protein RAD52. While a doctoral student, they also characterized a novel intrinsically disordered protein, DSS1, that regulates RAD52 activity and is upregulated in cancers. They also published three first author publications and contributed to two other manuscripts while at U Iowa.

Dr. Hengel came to UPMC Hillman Cancer Center in 2017 as a post-doctoral fellow in Dr. Kara Bernstein's laboratory. Their post-doctoral work has focused on understanding the role of the RAD51 paralog-containing complex, called the Shu complex, in yeast and human cells. Dr. Hengel dissected lesion specificity of the recombinant yeast Shu complex and found that this protein complex recognizes fork substrates containing abasic and 3MeC site lesions. This finding resulted in a co-first author publication in Nature Communications in 2019 and co-authorship on a follow-up manuscript in eLife in 2021.

Dr. Bernstein says, "Dr. Hengel has pioneered exciting new studies to uncover the mechanism of Shu complex function in promoting RAD51-mediated activities. Given that mutations in RAD51, and its regulators, are found in cancer, these findings will shed insight into how the Shu complex promotes genome stability and cancer prevention."

Dr. Hengel's current work is focused on the human Shu complex and its function in modulating RAD51-dependent activities using biochemical and single-molecule approaches. Their post-doctoral studies have been funded by a Diversity Supplement, the Hillman Cancer Center Postdoctoral Fellow for Innovative Cancer Research, an American Cancer Society Postdoctoral Fellowship, and a K99/R00 Grant from the NIEHS.

Dr. Hengel is a current collaborator of Dr. Carola Neumann (Pharmacology & Chemical Biology) and Drs. Yuan Chang, Patrick S. Moore, Patricia Opresko, and Ben Van Houten at UPMC Hillman Cancer Center.

Dr. Hengel has recently accepted a faculty position at Tufts University and will be starting her independent career fall of 2023. We wish her the best of luck.

Pitt Stop: Special Events and Visiting Speakers

Eric Greene

Eric Greene
Department of Biochemistry & Molecular Biophysics at Columbia University and also a member of the Herbert Irving Comprehensive Cancer Center (HICCC)

In person visit December 13-14, 2021
Contributed by Ben Van Houten, PhD

Dr. Eric Greene gave an outstanding hybrid seminar to a safely distanced audience and via Zoom, entitled, "Single Molecule Studies of Homologous Recombination." Professor Greene is an internationally recognized leader in the field using single molecule techniques to understand the assembly of recombination proteins to facilitate faithful exchange of genetic information during homologous recombination (HR). In a beautiful series of seminal papers, his group was able to show how Rad51 assembles on DNA, searches for homology and mediates strand transfer. Eric's lab developed a high throughput technique to watching single molecules using a novel DNA Curtain approach that greatly increased his through put and bandwidth, so over the years, he has characterized other recombination proteins at the single-molecule level including: BRCA1, BRCA2, Rad52, Rad54, as well as other DNA interacting proteins, including DNA helicases and even CRISPR/Cas 9 complexes. Dr. Greene's work is truly inspirational, and his papers are always a pleasure to read. His seminar highlighted a recent study on the role of RAD52 in HR, published in PNAS (2019), which due to COVID, he had not had an opportunity to present to an audience. The yeast Rad52 protein is known to help assemble Rad51 on single strand DNA to facilitate DNA strand exchange. During his seminar, Dr. Greene presented compelling data that Rad52 acts upstream of strand exchange by restricting DNA end processing at the site of the double-strand break. In the fission yeast, Rad52 was found to help select an Exo1 dependent limited resection over the extensive resection mediated by the helicase, Rdh1. In budding yeast, Rad52 similarly inhibits Sgs1 helicase dependent resection. This was shown directly using single molecule analysis that Rad52 specifically competes with Sgs1 for DNA end binding and inhibits Sgs1 translocation long DNA. Dr. Greene's presentation was well-received and generated a long series of questions. These results have important implications for stalled replication forks in human cells which occur during chemotherapy treatment, since RAD52 has been shown to protect stall forks from degradation.

Sharon Cantor

Sharon Cantor
Associate Research Director of the UMASS Cancer Center
Professor of Cancer Biology
University of Massachusetts Chan Medical School

Virtual visit February 14-15, 2022
Contributed by Patricia Opresko, PhD

Dr. Sharon Cantor gave a virtual seminar at the UPMC Hillman Cancer Center entitled "Rethinking BRCAness and Therapy Response." During her training with Dr. David Livingston, a pioneer in cancer genetics, Dr. Cantor discovered FANCJ helicase. Mutations in the FANCJ gene cause the genetic disorder Fanconi Anemia and are associated with hereditary breast and ovarian cancers. In her seminar, Dr. Cantor described exciting paradigm shifting work from her lab demonstrating that PARP inhibitors cause lethality in BRCA1 deficient cancer cells by generating single strand gaps, rather than by producing DNA double strand breaks. Her lab was the first to publish that toxic single strand gaps accumulate in BRAC1 deficient cells treated with PARP inhibitors, and this finding has since been reproduced in several studies from other laboratories. She presented evidence that gaps in BRAC1 deficient cells are due to defects in processing Okazaki fragments by using an elegant chromatin fiber assay and S1 nuclease to reveal single strand DNA gaps within replication tracts. She further described how suppression of gap formation is associated with resistance to PARP inhibitors. Finally, Dr. Cantor discussed clinical evidence for gaps and how to exploit gaps for enhancing cancer cell killing by genotoxic chemotherapy.

On Monday, prior to her seminar, Dr. Cantor delivered an outstanding lecture in the graduate course on Genome Instability and Human Disease about the chromosomal instability disorder Fanconi Anemia. She talked about an exciting time when two scientific fields merged, when researcher began discovering the same genes that, when mutated, cause hereditary forms of breast and ovarian cancer, and also cause Fanconi Anemia when both alleles are affected. She met with many of the GSP trainees after her lecture during an informal virtual lunch and shared her experiences and career advice. We are grateful to Dr. Cantor for taking the time to deliver two exciting lectures and to meet with many of our faculty and trainees.

Scientific Conference Highlights and Awards

1. **Gordon Research Seminar (GRS) and Conference (GRC) on Mammalian DNA Repair** October 31 to November 5, 2021

Several members of the Genome Stability Program attended the GRC on Mammalian DNA Repair, which was preceded by a GRS that was organized and focused on postdoctoral fellows and graduate students. Held in Ventura, CA, for many, this was the first in-person conference in a long time. The GSP was well-represented including:

- **Dr. Ragini Bhargava** co-chaired the GRS.
- **Dr. Mariarosaria De Rosa** presented "Roles for BER Enzymes in Telomeric 8-Oxoguanine Processing and Telomere Maintenance" at the GRS.
- **Dr. Namrata Kumar** presented "Global and Transcription-Coupled Repair of 8-OxoG Is Initiated by Nucleotide Excision Repair Proteins" at the GRS and was elected co-chair of the next GRS in 2023.
- **Dr. Patricia Opresko** served as Vice Chair of the GRC and led the Power Hour.
- **Dr. Sarah Hengel** presented "RAD51 Paralog Containing Complex SWSAP1-SWS1 Stimulates D-Loop Formation with Physiologically Relevant Substrate RPA Coated ssDNA" at the GRC.
- **Dr. Bennett Van Houten** presented "UV-DDB Functions to Stimulate Multiple DNA Glycosylases During Base Excision Repair of a Wide Variety of DNA Lesions: From Single-Molecules to Cells" in a special session at the GRC honoring base excision repair pioneer Sam Wilson.
- **Dr. Christopher Bakkenist** presented "Targeting ATM Deficiency in Cancer Therapy" at the GRC.
- **Dr. Elise Fouquerel** presented "PARP1 and PARP2 Prevent Oxidative Stress-Mediated Telomere Crisis Through Distinct Pathways" at the GRC.
- **Dr. Ryan Barnes** presented "Telomeric 8-Oxoguanine Drives Premature Senescence Independently of Telomere Shortening " at the GRC.
- **Spripriya Raja, Dr. Matt Schaich**, and **Sanjana Thosar** presented posters at the GRS and GRC.

2. **Dr. Van Houten** spoke at two recent international meetings: 1) the 11th Quinquennial Conference on Responses to DNA damage, Egmond aan Zee, The Netherlands, March 27 – April 01, 2022, and the 5th DNA Repair/Replication Structures and Cancer Conference, Cancun Mexico, April 27—May 1, 2022 , "Watching DNA repair in real time from single molecules to cells." **Brittani Schnable**, a graduate student in Dr. Van Houten's laboratory, won a poster award for her work on the stimulation of thymine DNA glycosylase by UV-DDB.
3. **Drs. Bakkenist, Bernstein,** and **Opresko** also spoke at the "sister" GRC on DNA Damage, Mutation, and Cancer held virtually March 6-11, 2022.
4. Cold Spring Harbor Laboratory meeting on Telomeres Telomerase
   December 31 to 17, 2021
   Several members of the Genome Stability Program attended the CHSL meeting on Telomeres & Telomerase, which was held virtually. Drs. Ryan Barnes and Elise Fouquerel also presented their work at this meeting, which included talks by the following GSP trainees.
   - **Dr. Samantha Sanford** presented "Investigating the impact of 6-thio-2'-deoxyguanosine on the telomerase catalytic cycle"
   - **Angela M. Hinchie** presented "Unexpected tolerance of variant telomere addition in a human pedigree".
5. Drs. Ryan Barnes and Sarah R. Hengel
   - Received a K99/R00 Fellowship (K99ES033738) from the NIEHS for their work on the human Shu complex and RAD52.
   - EMGS New Investigator Co-chair, an Early Career Reviewer for eLife, and a reviewer at DNA Repair
6. Dr. Yael Nechemia-Arbely
   - presented at the EpiCypher 2021 conference in November.
   - participated as an invited speaker at the 2021 The American Society of Cell Biology (ASCB) virtual conference and the Rising Star section of the Social DNAing webinar series, organized by Columbia University.
   - received the UPMC Health System Competitive Medical Research Fund (CMRF) in July 2021.

Hot Papers

1. Attaran, S., J. J. Skoko, B. L. Hopkins, M. K. Wright, L. E. Wood, A. Asan, H. A. Woo, A. Feinberg and C. A. Neumann (2021). "Peroxiredoxin-1 Tyr194 phosphorylation regulates LOX-dependent extracellular matrix remodelling in breast cancer." Br J Cancer **125**(8): 1146-1157.

2. Barroso-González, J., L. García-Expósito, P. Galaviz, M. L. Lynskey, J. A. M. Allen, S. Hoang, S. C. Watkins, H. A. Pickett and R. J. O'Sullivan (2021). "Anti-recombination function of MutSα restricts telomere extension by ALT-associated homology-directed repair." Cell Rep **37**(10): 110088.

3. Bonilla, B., A. J. Brown, S. R. Hengel, K. S. Rapchak, D. Mitchell, C. A. Pressimone, A. A. Fagunloye, T. T. Luong, R. A. Russell, R. K. Vyas, T. M. Mertz, H. S. Zaher, N. Mosammaparast, E. P. Malc, P. A. Mieczkowski, S. A. Roberts and K. A. Bernstein (2021). "The Shu complex prevents mutagenesis and cytotoxicity of single-strand specific alkylation lesions." Elife **10**.

4. Gardner, U. G., Jr., S. G. Wood, E. Y. Chen, J. S. Greenberger and A. J. Grossberg (2022). "Use of a Therapeutic Trial of Graduated Neoadjuvant Radiation Therapy for Locally Advanced Esophageal Cancer in a Patient With Fanconi Anemia." Adv Radiat Oncol **7**(1): 100810.

5. Hamsanathan, S., T. Anthonymuthu, S. Han, H. Shinglot, E. Siefken, A. Sims, P. Sen, H. L. Pepper, N. W. Snyder, H. Bayir, V. Kagan and A. U. Gurkar (2022). "Integrated -omics approach reveals persistent DNA damage rewires lipid metabolism and histone hyperacetylation via MYS-1/Tip60." Sci Adv **8**(7): eabl6083.

6. Hicks, K. C., Y. Y. Tyurina, V. E. Kagan and D. I. Gabrilovich (2022). "Myeloid Cell-Derived Oxidized Lipids and Regulation of the Tumor Microenvironment." Cancer Res **82**(2): 187-194.

7. Kumar, M., D. Molkentine, J. Molkentine, K. Bridges, T. Xie, L. Yang, A. Hefner, M. Gao, R. Bahri, A. Dhawan, M. J. Frederick, S. Seth, M. Abdelhakiem, B. M. Beadle, F. Johnson, J. Wang, L. Shen, T. Heffernan, A. Sheth, R. L. Ferris, J. N. Myers, C. R. Pickering and H. D. Skinner (2021). "Inhibition of histone acetyltransferase function radiosensitizes CREBBP/EP300 mutants via repression of homologous recombination, potentially targeting a gain of function." Nat Commun **12**(1): 6340.

8. Kumar, N., A. F. Theil, V. Roginskaya, Y. Ali, M. Calderon, S. C. Watkins, R. P. Barnes, P. L. Opresko, A. Pines, H. Lans, W. Vermeulen and B. Van Houten (2022). "Global and transcription-coupled repair of 8-oxoG is initiated by nucleotide excision repair proteins." Nat Commun **13**(1): 974.

9. Molkentine, D. P., J. M. Molkentine, K. A. Bridges, D. R. Valdecanas, A. Dhawan, R. Bahri, A. J. Hefner, M. Kumar, L. Yang, M. Abdelhakiem, P. M. Pifer, V. Sandulache, A. Sheth, B. M. Beadle, H. D. Thames, K. A. Mason, C. R. Pickering, R. E. Meyn and H. D. Skinner (2022). "p16 Represses DNA Damage Repair via a Novel Ubiquitin-Dependent Signaling Cascade." Cancer Res **82**(5): 916-928.

10. Paul, T., W. Liou, X. Cai, P. L. Opresko and S. Myong (2021). "TRF2 promotes dynamic and stepwise looping of POT1 bound telomeric overhang." Nucleic Acids Res **49**(21): 12377-12393.

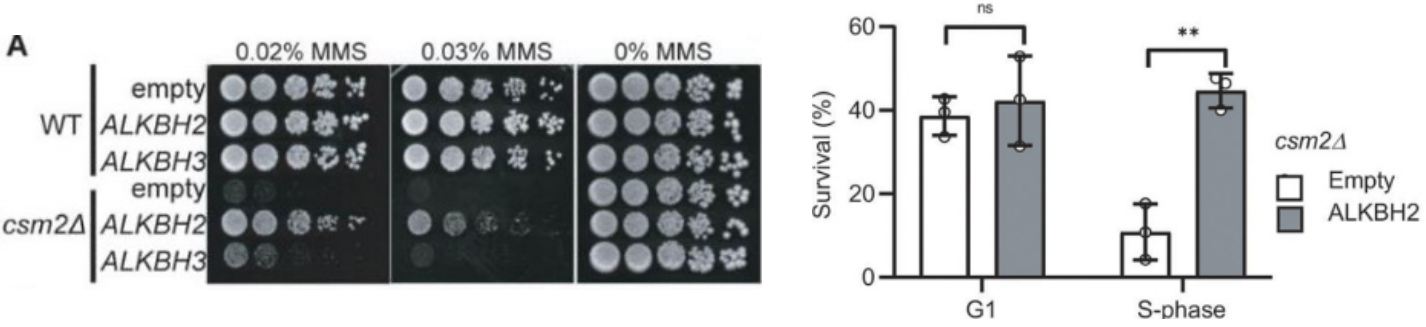
11. Tong, J., X. Tan, X. Song, M. Gao, D. Risnik, S. Hao, K. Ermine, P. Wang, H. Li, Y. Huang, J. Yu and L. Zhang (2022). "CDK4/6 inhibition suppresses p73 phosphorylation and activates DR5 to potentiate chemotherapy and immune checkpoint blockade." Cancer Res.

12. Vats, K., O. Kruglov, A. Mizes, S. N. Samovich, A. A. Amoscato, V. A. Tyurin, Y. Y. Tyurina, V. E. Kagan and Y. L. Bunimovich (2021). "Keratinocyte death by ferroptosis initiates skin inflammation after UVB exposure." Redox Biol **47**: 102143.

13. Wang, W., J. S. Prokopec, Y. Zhang, M. Sukhoplyasova, H. Shinglot, M. T. Wang, A. Linkermann, J. Stewart-Ornstein and Y. N. Gong (2022). "Sensing plasma membrane pore formation induces chemokine production in survivors of regulated necrosis." Dev Cell **57**(2): 228-245.e226.

14. Wilson, S. R. and A. W. Duncan (2022). "Single-Cell DNA Sequencing Reveals Chromosomal Diversity in HCC and a Novel Model of HCC Evolution." Gastroenterology **162**(1): 46-48.

15. Xu, J., X. Sun, K. Kim, R. M. Brand, D. Hartman, H. Ma, R. E. Brand, M. Bai and Y. Liu (2022). "Ultrastructural visualization of chromatin in cancer pathogenesis using a simple small-molecule fluorescent probe." Sci Adv **8**(9): eabm8293.



Expression of human ALKBH2 rescues the methyl methanesulfonate (MMS) sensitivity of csm2Δ cells. (Left) csm2Δ cells expressing ALKBH2 exhibit decreased MMS sensitivity. (Right) S-phase csm2Δ cells expressing ALKBH2 exhibit increased survival after acute MMS treatment. From: Bonilla, B., et al, Elife 10, 2021.

Cool Science

Anti-recombination function of MutSα restricts telomere extension by ALT-associated homology-directed repair.

Alternative lengthening of telomeres (ALT) is a telomerase-independent mechanism that nearly 15% of cancers rely on for telomere maintenance and continued proliferation. The ALT pathway uses mechanisms of homology-directed repair to extend shortened telomeres. In this international collaborative study, Dr. O'Sullivan and colleagues discovered that depletion of the MutSα (MSH2/MSH6) DNA mismatch repair (MMR) complex in ALT cells causes excessive telomere lengthening. This team uncovered evidence that MutSα restrains ALT by preventing recombination between heteroduplex sequences at telomeres, since ALT telomeres are known to contain variant telomere repeats. MutSα has a well-established function in MMR to correct DNA replication errors and prevent recombination between DNA sequences that lack perfect homology. This team further demonstrated that MutSα is recruited to telomeres by the proliferating-cell nuclear antigen, and that loss of MutSα led to enrichment of Bloom (BLM) helicase at telomeres. Depletion of BLM could suppress the hyper-telomere extension caused by MutSα loss, implicating BLM in the premature initiation of telomere extension. However, the simultaneous depletion of MutSα and BLM led to cell death in ALT cells.

Impact: This study uncovered a novel role for the mismatch repair complex MutSα in regulating telomere maintenance by the ALT pathway. ALT driven cancers are typically highly aggressive with poor prognosis, and the findings from this study that simultaneous loss of MutSα and BLM kill ALT cells has important therapeutic implications.

Funding: R01CA207209, R37CA263622, American Cancer Society RSG-18-038-01-DMC (R.J.O.) and 1S10OD019973 (S.C.W.), P30CA047904.

Barroso-González J, Garciá-Expósito L, Galaviz P, Lynskey ML, Allen JAM, Hoang S, Watkins SC, Pickett HA, O'Sullivan RJ. *Cell Report*, (2021). 37:110088. PMID: 34879271, PMCID: PMC8724847.

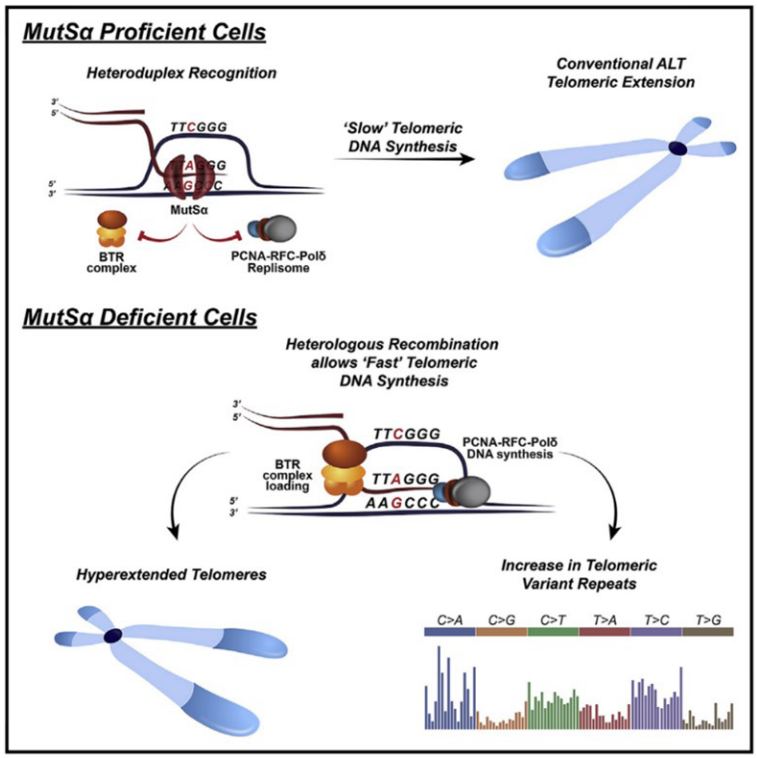


Figure Legend: Model for how MutSα restrains the recombination-based ALT pathways by preventing recombination between heteroduplex DNA and variant telomere repeat sequences. MutSα loss allows for premature initiation of telomere elongation involving the BLM helicase, leading to hyperextension of telomeres.

More Cool Science

Ultrastructural visualization of chromatin in cancer pathogenesis using a simple small-molecule fluorescent probe.

Imaging chromatin organization at the molecular-scale resolution remains an important endeavor in basic and translational research. Stochastic optical reconstruction microscopy (STORM) is a powerful super-resolution imaging technique to visualize nanoscale molecular organization down to the resolution of ~20 to 30 nm. Despite the substantial progress in imaging chromatin organization in cells and model systems, its routine application on assessing pathological tissue remains limited. It is, in part, hampered by the lack of simple labels that consistently generates high-quality STORM images on the highly processed clinical tissue. In this study Dr. Liu and her team developed a fast, simple, and robust small-molecule fluorescent probe—cyanine 5–conjugated Hoechst—for routine super-resolution imaging of nanoscale nuclear architecture on clinical tissue. They further demonstrated the significance of super-resolution imaging of molecular composition and structural characteristics of nuclear architecture in malignant transformation, which were not visible under conventional light microscopy. Their study demonstrated the biological and clinical significance of imaging super-resolved chromatin structure in cancer development and its potential clinical utility for cancer risk stratification.

Impact: These results not only demonstrated the robust performance of STORM imaging on clinical samples but also provided strong evidence for biological and clinical significance of imaging super-resolved nuclear architecture beyond conventional microscopic assessment in cancer research. Having a rapid and efficient method for based on light microscopy provides a new approach to stratifying cancer patients.

Funding: R01CA254112 (YL), R33CA225494 (YL), and R01CA232593 (YL) This project used the UPMC Hillman Cancer Center and Tissue and Research Pathology/Pitt Biospecimen Core shared resource, P30CA047904.

Xu, J., X. Sun, K. Kim, R. M. Brand, D. Hartman, H. Ma, R. E. Brand, M. Bai and Y. Liu (2022). Sci Adv 8(9): eabm8293.

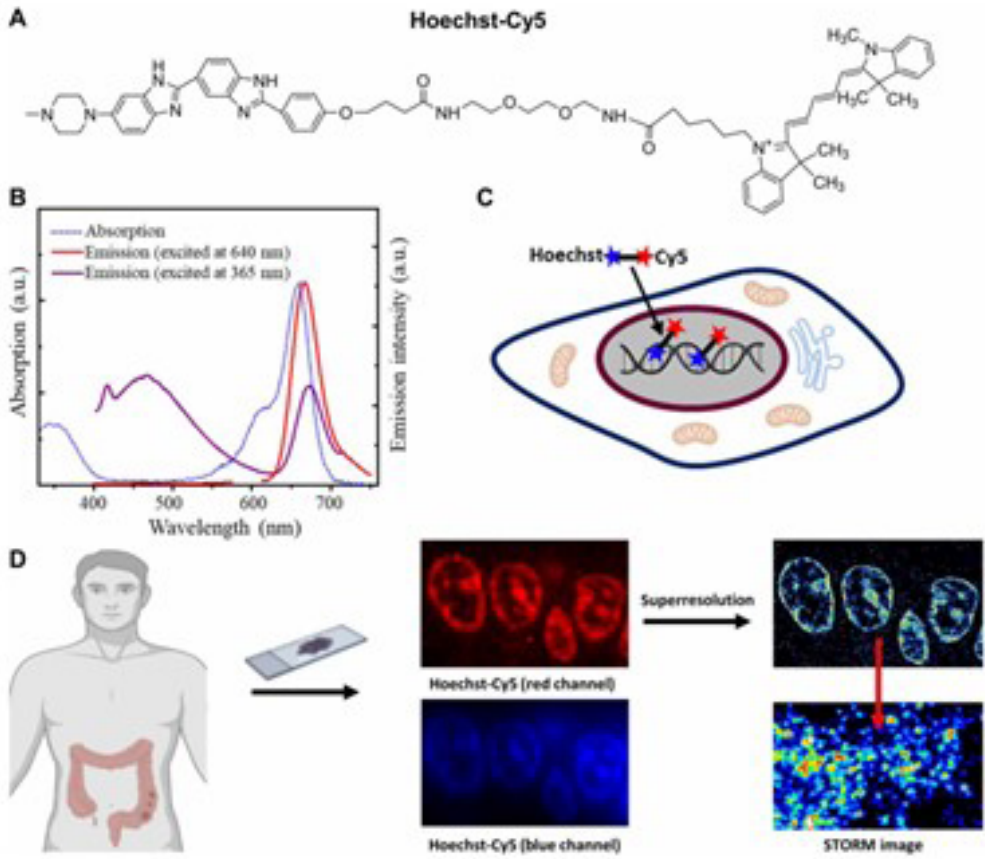


Figure legend: Schematic illustration of Hoechst-Cy5 for super-resolution imaging of genomic DNA on clinical tissue. (A) Chemical structure of Hoechst-Cy5. (B) Absorption spectra and emission spectra excited at 365 and 642 nm. a.u., arbitrary unit. (C) Schematic illustration of the Hoechst tagging strategy for DNA labeling with Cy5. (D) Schematic illustration of super-resolution imaging of chromatin structure on pathological tissue for disease assessment.

More Cool Science

Global and transcription-coupled repair of 8-oxoG is initiated by nucleotide excision repair proteins.

UV-DDB, consisting of subunits DDB1 and DDB2, recognizes UV-induced photoproducts during global genome nucleotide excision repair (GG-NER). Dr. Van Houten's group recently demonstrated a noncanonical role of UV-DDB in stimulating base excision repair (BER), which raised several questions about the timing of UV-DDB arrival at 8-oxoguanine (8-oxoG), and the dependency of UV-DDB on the recruitment of downstream BER and NER proteins. Using two different approaches to introduce 8-oxoG in cells, Drs. Opresko and Van Houten show that DDB2 is recruited to 8-oxoG immediately after damage and colocalizes with 8-oxoG glycosylase (OGG1) at sites of repair. 8-oxoG removal and OGG1 recruitment is significantly reduced in the absence of DDB2. NER proteins, XPA and XPC, also accumulate at 8-oxoG. While XPC recruitment is dependent on DDB2, XPA recruitment is DDB2-independent and transcription-coupled. Finally, DDB2 accumulation at 8-oxoG induces local chromatin unfolding. These data indicate that DDB2-mediated chromatin decompaction facilitates the recruitment of downstream BER proteins to 8-oxoG lesion.

Impact: This study demonstrates, for the first time, a convergence of several major DNA repair pathways in the removal of this highly mutagenic oxidative DNA lesion. Understanding the mechanism of 8-oxoG removal will provide insights into why certain patients with chronic pro-inflammatory oxidative stress may be more prone to tumors due to deficiencies in one of the proteins involved in the processing of this lesion.

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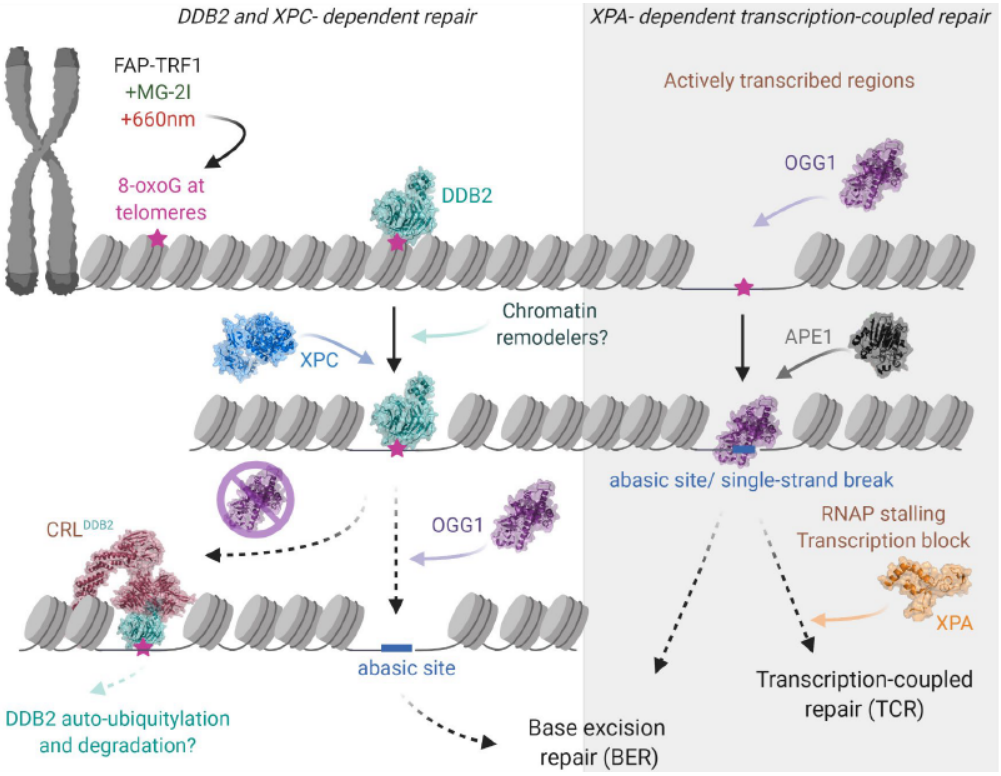

Figure Legend: Unified working model: role of NER proteins in 8-oxoguanine repair. Treatment of cells expressing FAP-TRF1 with dye (100 nM, 15 min) plus light (660 nm, 10 min) introduces 8-oxoG lesions at telomeres. In the DDB2-dependent repair pathway, DDB2 recognizes 8-oxoG lesions and facilitates chromatin relaxation through chromatin decompaction allowing the recruitment of XPC and OGG1 to the damage site. OGG1 recruitment facilitates the dissociation of DDB2. In the absence of downstream repair, DDB2 is retained longer at 8-oxoG sites requiring DDB1-Cul4A-RBX1 (CRL) mediated DDB2 dissociation. At actively transcribed regions, OGG1 can access the lesion independent of DDB2. 8-oxoG processing can lead to toxic BER intermediates that can act as a transcription block. Transcription-coupled repair (TCR) proteins, including XPA, participate in the repair of these BER intermediates.

More Cool Science

CDK4/6 inhibition suppresses p73 phosphorylation and activates DR5 to potentiate chemotherapy and immune checkpoint blockade.

Cyclin-dependent kinases 4 and 6 (CDK4/6) play a key role in cell cycle regulation by phosphorylating RB1 to relieve inhibition of E2F transcription factors and enable entry into S-phase. Inhibitors of CDK4/6 are used to treat breast and other solid tumors and act partly by halting cell cycle progression and promoting antitumor immunity. In this study Drs. Zhang and Yu, and colleagues sought to advance understanding of the antitumor activity of CDK4/6 inhibitors. This team discovered that CDK4/6 phosphorylates the p53 family member p73, thereby sequestering p73 to the cytoplasm. CDK4/6 inhibition led to p73 dephosphorylation and translocation to the nucleus, where p73 transcriptionally activated death receptor 5 (DR5), resulting in promotion of immunogenic cell death of cancer cells. Consistent with these findings, DR5 deletion in cancer cells both in vitro and in vivo abrogated the potentiating effects of CDK4/6 inhibitors in TNF-related apoptosis-inducing ligand, 5-fluorouracil chemotherapy, and anit-PD-1 immunotherapy.

Impact: This study demonstrates how CDK4/6 inhibitors sensitize cancer cells to chemotherapy and immune checkpoint blockade, suggesting DR5 induction may be a useful new molecular marker for improving CDK4/6-targeted cancer therapies.

Funding: R01CA203028, R01CA217141, R01CA236271, R01CA247231, and R01CA248112 (L.Z.), U19AI068021, R01CA215481 (J.Y.), R01CA260357 (Y.H.), T32GM133332 (K.E.), P30CA047904.

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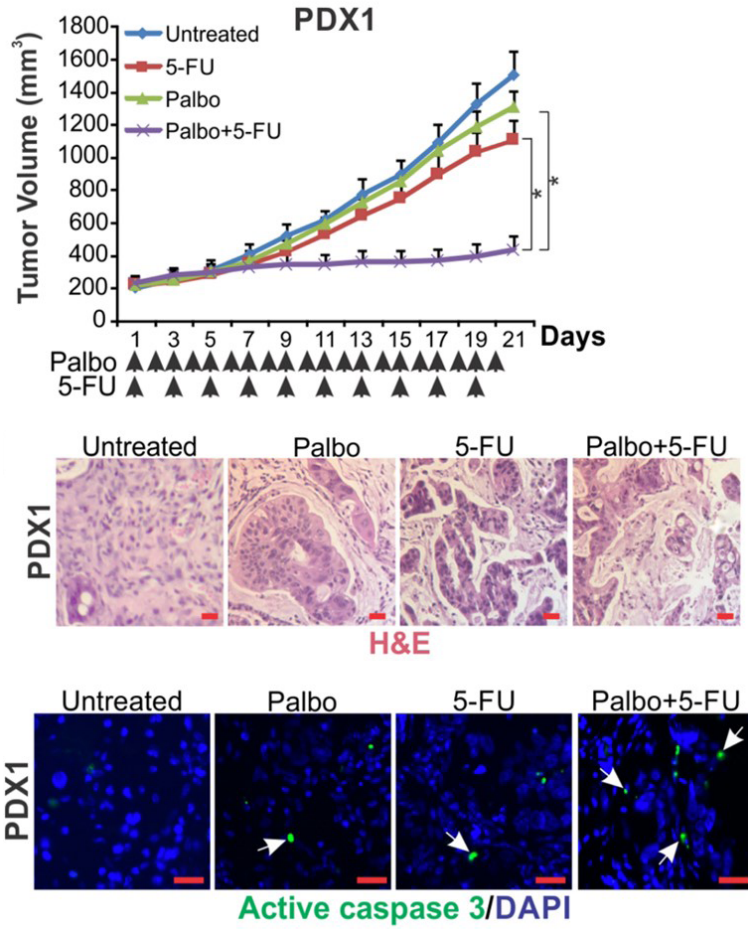

Figure Legend: Suppression of colorectal cancer cell tumor growth (patient derived xenograft, PDX1) in mice by CDK4/6 inhibitor (Palbo) combined with 5-FU. (F) Volume of PDX1 tumors at indicated times points. H&E staining (H) and active caspase 3 staining (J) of representative PDX1 tumors from mice treated for 10 days.

More Cool Science

Featured intra-programmatic collaboration:

Integrated -omics approach reveals persistent DNA damage rewires lipid metabolism and histone hyperacetylation via MYS-1/Tip60.

Persistent DNA damage drives numerous aging-related diseases including cancer, metabolic syndrome, cardiovascular disease and neurodegeneration. Survivors of pediatric cancers treated with chemotherapeutic DNA damaging drugs often exhibit premature frailty and aging-related pathologies in their mid-40s. In this intra-programmatic collaboration Drs. Gurkar, Bayir and Kagan investigated the underlying mechanisms of how genotoxic stress drives aging by using a DNA repair-deficient model of ERCC1-XPF in C. elegans. Using a multi-omics approach they discovered that nuclear DNA damage promotes mitochondrial β-oxidation and loss of fat depots in the worm. This metabolic shift generates acetyl-coenzyme A, leading to histone hyperacetylation and associated changes in expression of immune-effector and cytochrome genes. Histone acetyltransferase MYS-1 is a critical regulator of this metabolic-epigenetic axis, and loss of MYS-1 leads to an elevation in polyunsaturated fatty acids and arachidonic acid related mediators in response to DNA damage.

Impact: : These data reveal that nuclear DNA damage induces a metabolic shift to mitochondrial β-oxidation, altering the metabolic-epigenetic axis to drive an immune-like response that can promote aging-related pathologies. These findings have important implications for pediatric cancer survivors treated with genotoxic chemotherapeutics.

Funding: R00AG049126 (A.U.G.), R01GM132261 (N.W.S.), NIH ZIA AG000679 (P.S.), AI145406,CA165065, CA243142, AI068021, GM113908, HL114453, AI156924, NS076511, AI156923, andNS061817 (H.B. and V.K.).

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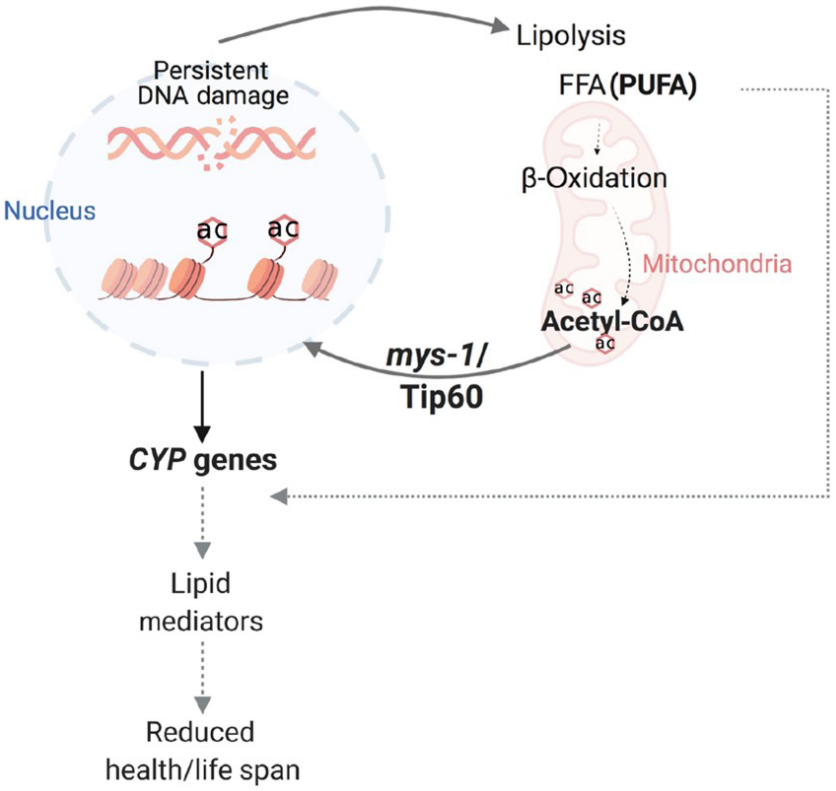

Figure legend: Model describing how persistent DNA damage drives increased mitochondrial B-oxidation to generate acetyl-CoA metabolite. Acetyl-CoA is used by the histone acetyltransferase (HAT), mys-1 (Tip60), to promote histone hyperacetylation leading to transcription of immune-effector and cytochrome (CYP) genes. PUFAs, such as arachidonic acid can be acted upon by CYP genes to generate AA-related lipid mediators and promote a "proinflammatory signature," affecting health and lifespan.

More Cool Science

Featured Clinical Study

Inhibition of histone acetyltransfrase function radiosensitizes CREBBP/EP300 mutants via repression of homologous recombination, potentially targeting a gain of function.

Despite radiation forming the curative backbone of over 50% of malignancies, there are no genomically-driven radiosensitizers for clinical use. Drs. Skinner's and Ferris' teams performed in vivo shRNA screening to identify targets generally associated with radiation response as well as those exhibiting a genomic dependency. They identified the histone acetyltransferases CREBBP/EP300 as a target for radiosensitization in combination with radiation in cognate mutant tumors. Further in vitro and in vivo studies confirm this phenomenon to be due to repression of homologous recombination following DNA damage and reproducible using chemical inhibition of histone acetyltransferase (HAT), but not bromodomain function. Selected mutations in CREBBP lead to a hyperacetylated state that increases CBP and BRCA1 acetylation, representing a gain of function targeted by HAT inhibition. Additionally, mutations in CREBBP/EP300 are associated with recurrence following radiation in squamous cell carcinoma cohorts. These findings provide both a mechanism of resistance and the potential for genomically-driven treatment.

Impact: These data reveal that gain of function mutations in CREBBP/EP300 can be targeted by a combination of ionizing radiation and HAT inhibition. The importance of CREBBP and EP300 mutation is underscored following analysis of tumor tissues in several cohorts of patients with SCC of the head and neck, lung, or cervix treated with radiation therapy, identifying these mutations as associated with radioresistance and poor outcome.

Funding: R01CA168485-08 (HS) and P50CA097190-15 (RF), R01 DE028105 (HS) and R01DE028061 (HS and CP), and The Cancer Prevention Institute of Texas RP150293 (HS and CP).

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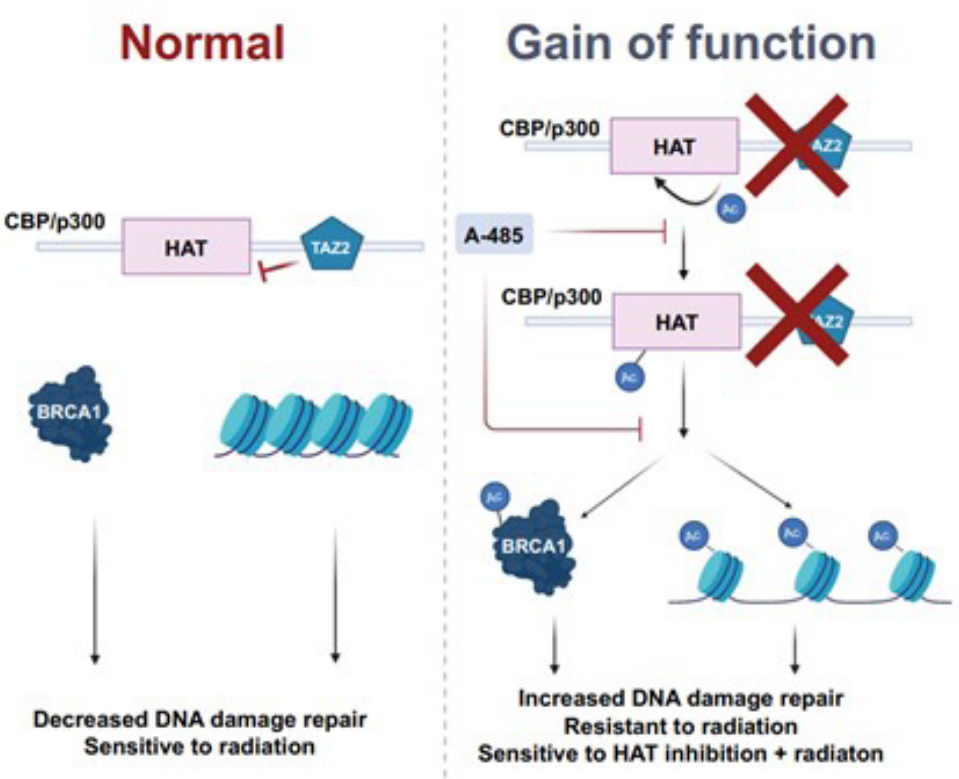

Figure legend: Proposed mechanism of CREBBP/EP300 mutant-specific radiosensitization. Normal functioning of CBP and p300 shown on the left, with a potential gain of function (GOF) for both proteins shown on the right. The GOF mutation generally leads to increased protein acetylation, particularly BRCA1 acetylation, and increased DNA damage repair. Cells harboring these GOF mutations are sensitive to the combination of histone acetyltransferase (HAT) inhibition and radiation.

Faculty and Staff News

Kara Bernstein, PhD

Farewell to **Kara Bernstein, PhD,** who will be moving to the University of Pennsylvania in the Department of Biochemistry and be associated with the Basser Center for BRCA at Penn Medicine's Abramson Cancer Center, founded by Mindy and Jon Gray. This is the first comprehensive center for the research, treatment, and prevention of BRCA-related cancers. The Center's unique model provides funding for collaborative research, education, and outreach programs around the world. Kara and her group have made many great contributions to the Genome Stability Program over the years. They will be sorely missed. We wish you and your team best of luck at Penn.

Welcome New Staff

Dr. Elise Fouquerel, PhD

Assistant Professor, Department of Pharmacology & Chemical Biology

University of Pittsburgh

Rim Nassar, PhD, postdoctoral associate

Daniela Muoio, PhD, postdoctoral associate

Natalie Laspata, PhD student

Lily Thompson, PhD student

The Fouquerel laboratory was established in December 2018 in the Department of Biochemistry and Molecular Biology at Thomas Jefferson University and joined the University of Pittsburgh Department of Pharmacology and Chemical Biology and UPMC Hillman Cancer Center in February 2022. They are interested in deciphering the roles of DNA-dependent ADP Ribose Transferase enzymes PARPs in the repair of oxidative DNA damage and resolution of secondary DNA structures at telomeres and centromeres, two genomic regions whose integrity is crucial for overall genome stability. They utilize biochemical, molecular, and cell biological tools to address their current questions. They also employ the FAP system, a highly innovative tool that allows for the local induction of oxidative DNA damage at the telomeres and the centromeres of mammalian cells. Their work contributes to improving the development of PARP inhibitors already largely used in cancer therapies and aims to provide new strategies for treating cancers and inform the rational design of PARP-targeted treatments.