Spring 2021

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 growing distribution of the COVID19 vaccine gives us hope that we are beginning to round the corner on the COVID19 pandemic. Amidst what has been an undeniably challenging year, the Genome Stability Program at the UPMC Hillman Cancer Center has continued to produce remarkable cancer research, evident in the work presented by their members at Hillman seminars, their recent publications and the consistency in grant funding. The GSP collaborates closely with members in other programs at Hillman to leverage our collective expertise to conduct impactful basic and translational research. An example of this collaborative approach is the Cancer and the Environment strategic vision team, which is co-led by Dr. Patty Opresko from the GSP and Dr. Jian-Min Yuan, co-leader of the program on Cancer Epidemiology and Prevention.

The DNA Pitt Crew newsletter gives me an opportunity to learn about exceptional science happening at Hillman and the remarkable people behind it. I take great pleasure in reading it and I hope you will too.

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

We are very pleased to present the Spring 2021 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. Living and working under the constraints of the COVID-19 pandemic continues to be a challenge for all of us. Despite everyone's willingness to meet virtually, "Zoom fatigue" is setting in, and we look forward to herd immunity when we can all meet face to face. 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. In our "hot papers" section, we are delighted to present recent impactful papers published in Nature Structural & Molecular Biology, eLife, Nature Communications, JCI Insight, and Science Advances, among others. Our description of this "cool science" points out important inter-programmatic collaborations of GSP members with other program members. Our work-in-progress seminar series on Wednesday mornings from 9 to 10 a.m. continues to thrive with 40-50 in virtual attendance. These seminars are great opportunities to share unpublished data and discuss publication models with a wider audience. As part of a strategic working group on Cancer and the Environment, Patty Opresko led an exciting half-day workshop, which was started by an exceptional keynote lecture by Dr. Ludmil Alexandrov entitled, "The Repertoire of Mutational Signatures in Human Cancer." This tour-de-force seminar provided an outstanding overview of how Dr. Alexandrov and his team were able to extract mutational signatures in specific cancer types. This genetic archeological profiling of tumors gives a history of genetic changes and potential environmental genotoxicants that caused specific mutation spectra, contributing to the rise of the neoplastic lesion. We were especially fortunate to have Dr. Alexandrov provide insightful comments through the workshop that sought to understand the environmental factors causing an increased risk of lung cancer, especially among non-smokers. Such environmental factors include the high levels of radon and air pollution in our catchment area in Western Pennsylvania. As part of director Dr. Robert Ferris' initiative to bring "disruptive technology" to our Cancer Center, a group of scientists, led by Dr. Van Houten, with the generous support of HCC were able to lease a Lumicks C-Trap® instrument optical platform that combines optical tweezers, three-color confocal microscopy, and microfluidics with a five-chamber flow cell to analyze single molecules in real-time. We are eager to work with other HCC scientists exploring the full power of this highly innovative instrument in the coming months to address fundamental questions in cancer biology.

Training opportunities:
Please see Hillman Postdoctoral Fellows for Innovative Cancer Research (page 10).

Inside this issue:

Contact Us:

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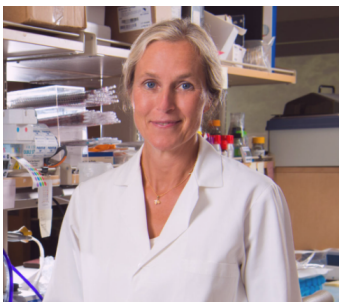


Carola Neumann, MD

Faculty Spotlight: Carola Neumann, MD

Dr. Carola Neumann is a German native from the lovely state of Bavaria in the south of Germany. Dr. Neumann was initially trained as a clinical laboratory assistant and worked then for a year in the Kantonspital in Basel, Switzerland, before going to medical school at the Ludwig Maximilian University in Munich. Towards the end of her training, she obtained a fellowship from the Deutsche Akademischer Austauschdienst (DAAD) to finish her medical thesis "IL-6 induced signaling in multiple myeloma" at the Brigham and Women's Hospital (BWH) at Harvard Medical School (HMS) in Boston, MA and to spend her final practical year of medical school at Boston University and Brown University in Rhode Island. Her heart for research, however, kept beating, and after being offered a postdoctoral fellowship at HMS, she started working in the laboratory of Dr. Rick VanEtten on chronic myeloid leukemia and the BCR-ABL inhibitory protein peroxiredoxin 1 (PRDX1). During her first postdoctoral fellowship, Dr. Neumann gave birth to three lovely children (Paulina and then twins: Jule and Gretchen) and was fortunate to obtain an F32 Postdoctoral Individual National Research Service Award (Ruth L. Kirschstein) from the NIEHS. From the VanEtten lab she continued her postdoctoral training with Dr. Piotr Sicinski at the Dana Farber Cancer Institute (DFCI), where she got first exposed to breast cancer research and received her K22 award from the NIEHS. In 2006, Dr. Neumann moved with her family to Charleston, SC, to begin her Assistant Professorship in the Dept. of Pharmacology and Experimental Therapeutics. There, she continued the study of peroxiredoxin 1 in breast cancer development. At MUSC, she founded a breast cancer research program for which she obtained training funding from the DoD, and also secured several research awards from the DoD and the NCI. Dr. Neuman had her 4th child, Lukas, in Charleston. In 2012, Dr. Neumann joined the Dept. of Pharmacology and Chemical Biology at the University of Pittsburgh. PITT offered the unique opportunity to combine the three research interests of the Neumann lab, namely redox cancer biology, breast cancer and DNA DSB repair. At PITT, Dr. Neumann has continued her study of peroxiredoxin 1, and started together with Drs. Schopfer and Freeman (Dept. of Pharmacology and Chemical Biology), a drug development program advancing the use of nitroalkenes as inhibitors of DNA DSB repair. This effort produced successful IP protection and funding of Creegh Pharmaceuticals. Besides her research efforts, Dr. Neumann has also been co-leading the breast cancer research advocacy network (bcRAN) since 2013 at UPMC Magee Women's Hospital and UPMC Hillman Cancer Center. This group of breast cancer survivor advocates has educated the community in Allegheny county about the importance of breast cancer research and clinical trials through intense outreach work. Dr. Neumann has continued to obtain funding for research and advocacy from Komen, Magee Foundation, DoD and NCI. She is currently a tenured Associate Professor and the newly appointed Vice-Chair of Precision and Translational Pharmacology in the Dept. of Pharmacology and Chemical Biology.

Braulio Bonilla Villagran

Trainee Spotlight: Braulio Bonilla Villagran (Bernstein Lab)

Contributed by: Braulio Bonilla, PhD and Kara Bernstein, PhD

Dr. Bonilla is from Minas, Uruguay. He graduated from Universidad de la República with a degree in Biology. As an undergrad, he studied the genetic diversity of the Canine Distemper virus in Uruguay. He then obtained a Master's degree in Molecular and Cell Biology at the Institut Pasteur of Montevideo. During this time, his research work focused on the regulation of the tumor suppressor CHD5 by microRNAs. Always driven by the desire of experiencing new things, he decided to continue with his graduate studies abroad and join the Bernstein Lab to pursue his PhD.

Dr. Bonilla quickly became fascinated with the yeast model and focused his dissertation on understanding the homologous recombination factor's role, the Shu complex, in the tolerance of alkylation DNA damage. Using a combination of genetics, deep sequencing, and molecular biology, Dr. Bonilla dissected the lesion specificity of the Shu complex. His results propose that the Shu complex is crucial for the error-free tolerance of abasic sites and 3-methylcytosines. His work led to a first author publication in Nature Communications, a review article in Annual Reviews of Genetics, and a manuscript currently submitted to Cell Reports. Dr. Bonilla defended his doctoral dissertation and graduated from the Molecular Pharmacology Graduate Student Program in December of 2020.

Dr. Bonilla is currently a postdoctoral fellow at Dr. Sarah Hainer Lab, where he aims to elucidate the role of enhancer and promoter RNA on gene regulation in embryonic stem cells.

Dr. Bernstein says, "Braulio was an exemplary graduate student. He is a deep thinker who is able to unravel complex ideas into testable hypotheses. I'm extremely proud of his accomplishments in the lab and his discovery of a new role for the Shu complex in identifying and bypassing abasic sites to protect replication forks from double-strand break formation. I look forward to watching him progress during his postdoctoral training to an independent scientist and future colleague."

Pitt Stop: Special Events and Visiting Speakers

Karlene Cimprich, PhD
Professor and Vice-Chair of Chemical and Systems Biology
Stanford University School of Medicine

Virtual visit December 1, 2020
Contributed by Ryan Barnes, PhD

Karlene Cimprich

Dr. Karlene Cimprich gave a virtual seminar at the UPMC Hillman Cancer Center entitled "The Causes and Consequences of Replication Stress." Dr. Cimprich has been a well-recognized expert in the field of DNA replication stress since she discovered human ATR kinase, a key regulator of the replication stress response and a current target in many clinical trials for cancer therapy. On Dec 1, Dr. Cimprich gave a recap of her laboratory's recent exciting publication in Molecular Cell, in which they investigated the role of HLTF protein in promoting replication fork reversal during replication stress and the role of primase-polymerase PRIMPOL in fork elongation. The talk then shifted to discussing unpublished work concerning RNA/DNA hybrids (R-loops). Using a modification of an established assay for isolating and sequencing these structures, Dr. Cimprich's lab found they existed not only in the nucleus but also in the cytoplasm of human cancer cells. Intriguingly, these cytoplasmic R-loops are somewhat resistant to degradation by RNAseH and could stimulate immune signaling. In addition to meeting with various professors at the Hillman, Dr. Cimprich also chatted with Postdoctoral fellows and Graduate students. She was very interested in our projects, our experiences working at the Hillman, and how we handled working during the COVID-19 pandemic, and she provided valuable feedback.

Western Pennsylvania (WPA) Environmental Risk Factors for Lung Cancer Brainstorming Mini-Workshop on Mutational Signatures of Exposures

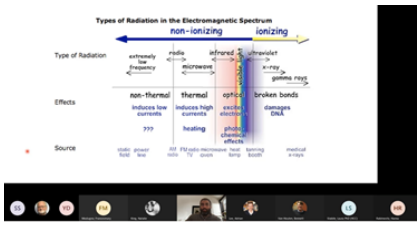
Held February 9, 2021

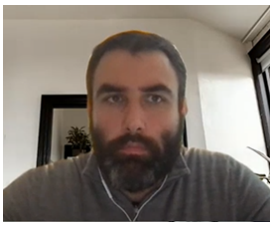
Contributed by Patricia L. Opresko, PhD

This workshop was sponsored by the UPMC Hillman Cancer Center and organized by the HCC strategic vision team on Cancer and Environment, chaired by Drs. Patricia Opresko and Jian-Min Yuan. The goals of this team are to work together to develop multidisciplinary research projects with basic, translational and education outreach components that address environmental risk factors for cancer in western PA. Next-generation sequencing has led to the identification of somatic mutations in cancer genomes, caused by multiple mutational processes and exposures which generate characteristic mutational signatures. The specific goals of this mini-workshop were to explore use of mutational signatures in clinical samples to:

1. Inform cancer etiology and contribution of environmental exposures
2. Develop hypotheses for an increase in non-smoking-related lung tumors
3. Inform strategies for precision medicine
4. Determine other potential uses and applications

We were privileged to have Ludmil Alexandrov, PhD, Assistant Professor, Cellular and Molecular Medicine, University of California San Diego, kick off the event with an outstanding keynote lecture entitled "The Repertoire of Mutational Signatures in Human Cancer". During his PhD work, Dr. Alexandrov published the first comprehensive map of the signatures of mutational processes that cause somatic mutations in human cancer. In his lecture, he summarized his studies identifying distinct mutational signatures in tumor genomes of various environmental exposures including UV light, tobacco smoke, and aristolochic acid, along with various chemotherapeutic drugs, including temozolomide and azathioprine. He also presented evidence for defining mutational signatures caused by defects in specific DNA repair and replication enzymes. He then participated as an expert panelist in three sessions, which consisted of short presentations followed by questions and answers related to the





potential use of mutational signatures in defining cancer etiology in western PA lung cancers. These sessions were highly engaging, and we thank all the participants for lively discussions, especially Dr. Alexandrov, for sharing his expertise and addressing all our questions.

The following workshop sessions included HCC speakers and panelists:

The Pittsburgh Lung Cancer Program Study Populations, presented by Laura Stabile, PhD, Associate Professor, Pharmacology and Chemical Biology.

Panelists: Laura Stabile, PhD; James Herman, MD, Professor of Medicine; Timothy Burns, MD, PhD, Assistant Professor of Medicine; Brenda Diergaarde, PhD, Associate Professor, Human Genetics.

Radon exposure in Pittsburgh and WPA, presented by Shaina Stacy, PhD, Postdoctoral Fellow.

Panelists: Shaina Stacy, PhD; Jian-Min Yuan, MD, PhD, Professor of Epidemiology; Nate Burden, Physicist, President PA Chapter of the American Associate of Radon Scientists and Technicians

NGS facilities and capabilities at UPMC Hillman Cancer Center, presented by Adrian Lee, PhD, Director of the Institute for Precision Medicine, Professor of Pharmacology and Chemical Biology

Panelists: Riyue Bao, PhD, Research Associate Professor of Medicine, Co-Director of Bioinformatics; Annerose Bernt, PhD, DVM, Co-Director of Hillman Cancer Genomics Facility; Yuri Nikiforov, MD, PhD, Professor of Pathology, Co-Director of the Hillman Cancer Genomics Facility; Xiaosong Wang, MD, PhD, Professor of Pathology

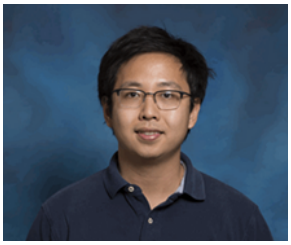
Scientific Conference Highlights and Awards

The Environmental Mutagenesis and Genomics Society Webinar and Online Workshop (WOW): Genome Instability and Disease

February 16, 2021

This webinar was the first EMGS DNA Repair Special Interest Group WOW event, which consisted of a half-day symposium designed to bring together both experts and newcomers to the field of DNA repair. The Keynote talk was given by Dr. Sylvie Doublie (University of Vermont), an expert in Structural Biology of DNA Repair Enzymes. This was followed by a workshop focused on current cell-based approaches for targeted DNA damage. The event ended with a series of eight outstanding talks covering a wide range of interests related to DNA repair and genome stability and included three GSP trainees.

- **Luong Thong (PhD student Bernstein lab):** Regulation of budding yeast Hrq1 helicase during DNA crosslink repair
- **Ryan Barnes, PhD (postdoctoral fellow Opresko lab):** Telomeric 8-oxoguanine drives premature senescence independently of telomere shortening
- **Sarah Hengel, PhD (postdoctoral fellow Bernstein lab):** The BRCA2-interacting protein, PDS5B, and SPIDR are novel Shu complex components



Luong Thong

Ryan Barnes

Sarah Hengel

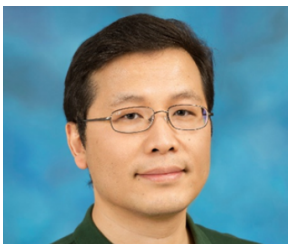
Congratulations Heath Skinner, MD, PhD

Congratulations to **Heath Skinner, MD, PhD,** who was elected to the American Society for Clinical Investigation. The American Society for Clinical Investigation seeks to support the scientific efforts, educational needs, and clinical aspirations of physician-scientists to improve the health of all people. The ASCI is a member society of the Federation of American Societies for Experimental Biology and Research!America. These organizations promote increased federal funding and sound science policy through direct Congressional advocacy and member participation.

Heath Skinner

Congratulations Lin Zhang, PhD

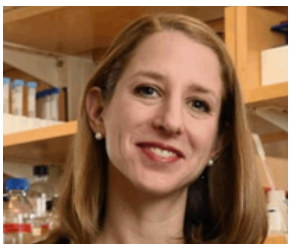
Lin Zhang, PhD: New NIH/NCI **R01 CA248112-A1** grant entitled "**BET degraders for improving colorectal cancer therapy."** This project is aimed at developing improved colon cancer therapies by inducing degradation of BET (bromodomain and extra-terminal domain) family proteins using compounds designed by PROTAC (Proteolysis Targeting Chimeras) strategy. The proposed studies will provide key preclinical data, new mechanistic insight, biomarkers of sensitivity and resistance, and rationales for effective combinations for developing BET degrader personalized therapies against therapy-refractory colon cancers.



Lin Zhang

Congratulations Kara Bernstein, PhD

Kara Bernstein, PhD: New NIH/NIEHS **R01 ES031796-01A1** grant entitled **"RAD51 paralog function in cancer predisposition and genome integrity."** The RAD51 paralogs are tumor suppressors, and women with breast or ovarian cancer are now screened for RAD51 paralog mutations. However, it remains largely unknown which RAD51 paralog mutations are pathogenic and how these mutations sensitize individuals to environmentally induced-DNA damage. This grant will use complementary approaches in combination with high-throughput genetic screening to address how RAD51 paralog mutations predispose individuals to human cancer and thus, to identify opportunities for determining who is at risk for cancer development upon exposure to environmental carcinogens.



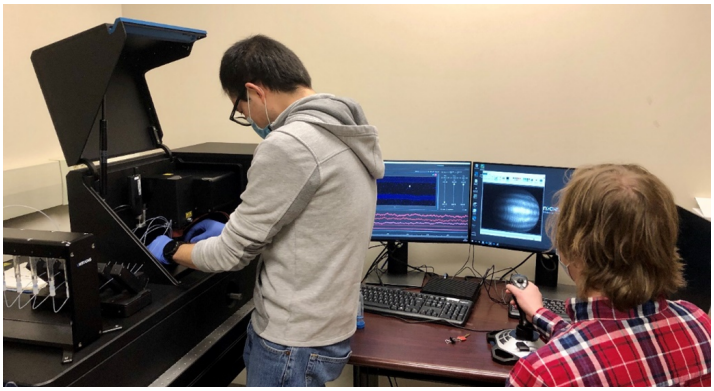
Kara Bernstein

Hillman Cancer Center Enables New Science

Lumicks C-Trap® Optical tweezers fluorescence microscope imaging system

The UPMC Hillman Cancer Center leases a new instrument to allow scientists to follow single molecules in action.

Contributed by Matt Schaich, PhD and Zhou Zhong, PhD



Drs. Zhou Zhong (left) and Matt Schaich (right) set-up the C-Trap® for collecting data.

Due to the generosity of Hillman Cancer Center, faculty at the HCC recently added an exciting new instrument to their toolbox for investigating single-molecule interactions, the C-Trap® from Lumicks. This highly innovative tool consists of an integrated system:

1. A five-channel microfluidic flow cell
2. A confocal imaging module
3. Two optical tweezers
4. A dedicated software analysis tool

The optical tweezers on C-Trap® can capture and apply precise forces to objects as small as 1 µm beads – when these beads are coated with a linking molecule like streptavidin, any molecule that can be biotinylated can be trapped. One reason that trapping is important is that it can reduce the movement of fluorophores, resulting in a clearer image of their position. By applying tension to its traps, the C-Trap® can achieve an incredible localization accuracy of 10 nm!

Because there are two separate optical tweezers on the instrument, this experimental setup is perfect for stringing up macromolecules such as DNA and RNA between the optical traps. With a known DNA length, the C-Trap® can detect a single strand of unlabeled DNA linked between the beads by comparing the force measurements on the beads (at sub-picoNewton resolution) with the distance between them. With a single piece of DNA "in hand", it can be moved between channels in the flow cell to study the way that proteins interact with DNA at the single-molecule scale, including:

- The locations along the DNA where proteins bind
- The distances proteins move after they bind DNA
- The way that multiple proteins work together (using the C-Trap's three-color fluorescence)

We in the Van Houten group have successfully utilized the C-Trap® to study UV-DDB, an important DNA damage recognition protein that, when mutated, can cause skin cancer. We observed hundreds of Cy3-labeled UV-DDB molecules dancing on a single UV-irradiated lambda DNA molecule over an hour. Furthermore, we saw that after one molecule of UV-DDB stably bound the damaged DNA, it would leave before another molecule came in to bind in the DNA at the exact same place stably. This proof-of-concept indicates that the C-Trap® can be used to interrogate specific protein-DNA interactions and allow rapid screening of protein variants associated with cancer. Furthermore, the ability to track three colors with precise force controls should allow the reconstitution of molecular processes in real-time. So, if you think your protein of interest could be better understood with this revolutionary technology, come see for yourself the power of this new C-Trap® instrument and become a "C-trapper."

Newly minted "C-trappers" in the atrium of the UPMC Hillman Cancer Center, after a fun week of training by Lumick's Application Specialist, Evan Gates (far right). Back row (left to right): James Li, Brittani Schnable, Samantha Sanford, Namrata Kumar, Zhou Zhong, Matt Schaich, Shenyu Sun, Ben Van Houten, and Evan Gates.

Front Row (left to right): Yuan Chang and Sarah Hengel.

Hot Papers

Anthonymuthu TS, Tyurina YY, Sun WY, Mikulska-Ruminska K, Shrivastava IH, Tyurin VA, Cinemre FB, Dar HH, VanDemark AP, Holman TR, Sadovsky Y, Stockwell BR, He RR, Bahar I, Bayır H, Kagan VE. Resolving the paradox of ferroptotic cell death: Ferrostatin-1 binds to 15LOX/PEBP1 complex, suppresses generation of peroxidized ETE-PE, and protects against ferroptosis. Redox Biol 38: 101744. doi: 10.1016/j.redox.2020.101744. Epub 2020 Oct 16. PMID: 33126055; PMCID: PMC7596334.

Chen SY, Osimiri LC, Chevalier M, Bugaj LJ, Nguyen TH, Greenstein RA, Ng AH, Stewart-Ornstein J, Neves LT, El-Samad H. Optogenetic Control Reveals Differential Promoter Interpretation of Transcription Factor Nuclear Translocation Dynamics. Cell Syst. 2020 Oct 21;11(4):336-353.e24. doi: 10.1016/j.cels.2020.08.009. Epub 2020 Sep 7. PMID: 32898473; PMCID: PMC7648432.

Hoang SM, Kaminski N, Bhargava R, Barroso-González J, Lynskey ML, García-Expósito L, Roncaioli JL, Wondisford AR, Wallace CT, Watkins SC, James DI, Waddell ID, Ogilvie D, Smith KM, da Veiga Leprevost F, Mellacharevu D, Nesvizhskii AI, Li J, Ray-Gallet D, Sobol RW, Almouzni G, O'Sullivan RJ. Regulation of ALT-associated homology-directed repair by polyADP-ribosylation. *Nat Struct Mol Biol*. 2020 Dec;27(12):1152-1164. PMID: 33046907

Houston R, Sekine S, Calderon MJ, Seifuddin F, Wang G, Kawagishi H, Malide DA, Li Y, Gucek M, Pirooznia M, Nelson AJ, Stokes MP, Stewart-Ornstein J, Mullett SJ, Wendell SG, Watkins SC, Finkel T, Sekine Y. Acetylation-mediated remodeling of the nucleolus regulates cellular acetyl-CoA responses. PLoS Biol. 2020 Nov 30;18(11):e3000981. doi: 10.1371/journal.pbio.3000981. PMID: 33253182; PMCID: PMC7728262.

Liu CH, Chen Z, Chen K, Liao FT, Chung CE, Liu X, Lin YC, Keohavong P, Leikauf GD, Di YP. Lipopolysaccharide-Mediated Chronic Inflammation Promotes Tobacco Carcinogen-Induced Lung Cancer and Determines the Efficacy of Immunotherapy. Cancer Res. 2021 Jan 1;81(1):144-157. doi: 10.1158/0008-5472. CAN-20-1994. Epub 2020 Oct 29. PMID: 33122306; PMCID: PMC7878420.

Ruan H, Li X, Xu X, Leibowitz BJ, Tong J, Chen L, Ao L, Xing W, Luo J, Yu Y, Schoen RE, Sonenberg N, Lu X, Zhang L, Yu J eIF4E S209 phosphorylation licenses myc- and stress-driven oncogenesis. *Elife*. 2020 Nov 2;9:e60151. PMID: 33135632

Sanford S, Welfer G, Freudenthal B, and Opresko PL. Mechanisms of telomerase inhibition by oxidized and therapeutic dNTPs."*Nature Communications*, 2020 Oct 20; 11(1):5288. PMID: 33082336, PMCID: PMC7576608.

Stabile LP, Kumar V, Gaither-Davis A, Huang EH, Vendetti FP, Devadassan P, Dacic S, Bao R, Steinman RA, Burns TF, Bakkenist BJ. Syngeneic tobacco carcinogen-induced mouse lung adenocarcinoma model exhibits PD-L1 expression and high tumor mutational burden. *JCI Insight*, 2021;6(3):e145307. PMID: 33351788

Toptan T, Cantrell PS, Zeng X, Liu Y, Sun M, Yates NA, Chang Y, Moore PS. Proteomic approach to discover human cancer viruses from formalin-fixed tissues. JCI Insight. 2020 Nov 19;5(22):e143003. doi: 10.1172/jci.insight.143003. PMID: 33055416; PMCID: PMC7710300.

Uzunparmak B, Gao M, Lindemann A, Erikson K, Wang L, Lin E, Frank SJ, Gleber-Netto FO, Zhao M, Skinner HD, Newton J, Sikora AG, Myers JN, Pickering CR. Caspase-8 loss radiosensitizes head and neck squamous cell carcinoma to SMAC mimetic-induced necroptosis. JCI Insight. 2020 Dec 3;5(23):e139837. doi: 10.1172/jci.insight.139837. MID: 33108350; PMCID: PMC7714407.

Cool Science

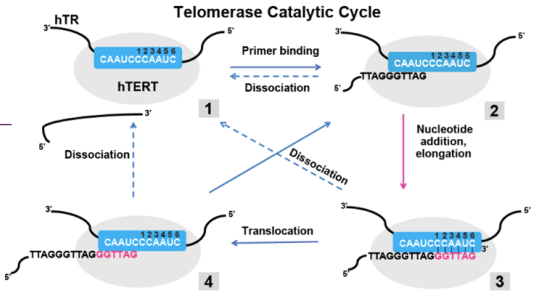
Mechanisms of telomerase inhibition by oxidized and therapeutic dNTPs.

Telomerase is a specialized reverse transcriptase that adds GGTTAG repeats to chromosome ends and is upregulated in most human cancers to enable limitless proliferation. In this study, Dr. Opresko and colleagues uncovered two distinct mechanisms by which naturally occurring oxidized dNTPs and therapeutic dNTPs inhibit telomerase-mediated telomere elongation. Through a series of biochemical telomerase extension assays in the presence of modified dNTPs, they provide direct evidence that telomerase can add the HIV nucleotide reverse transcriptase inhibitor drugs ddITP and AZT-TP to the telomeric end, causing chain termination. In contrast, telomerase continues elongation after inserting oxidized 2-OH-dATP or therapeutic 6-thio-dGTP, but insertion disrupts translocation and inhibits further repeat addition. Kinetic studies reveal that telomerase poorly selects against 6-thio-dGTP, resulting in a very low IC50 for telomerase extension.

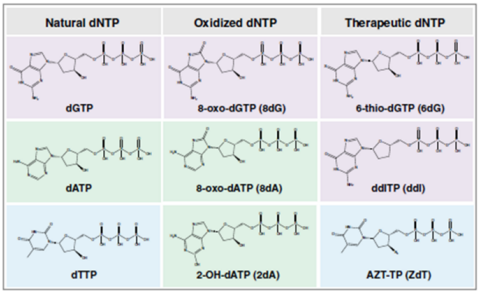
Impact: Findings from this study have important health implications for potential off-target effects on long-term nucleoside reverse transcriptase inhibitors treatments for HIV infections and for therapeutic strategies to target telomerase in cancer to halt proliferation. These results explain how 6-thio-dG treatments cause telomere shortening and dysfunction, as well as reduced tumor growth, in mouse models.

Funding: R01CA207342 and R35030396 (to P.L.O.), and P30CA047904.

Sanford S, Welfer G, Freudenthal B, and Opresko PL. *Nature Communications,* 2020 Oct 20; 11(1):5288. PMID: 33082336, PMCID: PMC7576608.



Schematic of the telomerase catalytic cycle. Blue indicates the telomerase RNA template; black indicates DNA primer; red indicates newly added nucleotides. Modified dNTPs that cause chain termination inhibit step 2, whereas modified dNTPs that disrupt telomerase translocation inhibit steps 3.



More Cool Science

Syngeneic tobacco carcinogen-induced mouse lung adenocarcinoma model exhibits PD-L1 expression and high tumor mutational burden.

Human lung adenocarcinoma (LUAD) in current or former smokers exhibits a high tumor mutational burden (TMB) and distinct mutational signatures, however, immune-competent models of clinically relevant smoking-related LUAD are lacking. To address this, Dr. Stabile (Cancer Biology Program) and colleagues, including Dr. Bakkenist (Genome Stability Program), established and characterized a tobacco-associated, transplantable murine LUAD cell line, designated FVBW-17, from a LUAD induced by the tobacco carcinogen NNK. Whole-exome sequencing of these cells identified tobacco-associated KrasG12D and Trp53 mutations and a similar mutation profile to that of classic alkylating agents with a TMB greater than 500. FVBW-17 cells transplanted subcutaneously and orthotopically in mice generated tumors that were histologically similar to human LUAD. These tumors also expressed programmed death ligand 1 (PD-L1), were infiltrated with CD8+ T cells, and were responsive to immune checkpoint inhibitor anti–PD-L1 therapy. FVBW-17 cells expressing green fluorescent protein and luciferase enabled quantification of tumor growth, and of distant metastases to lung, spleen, liver, and kidney.

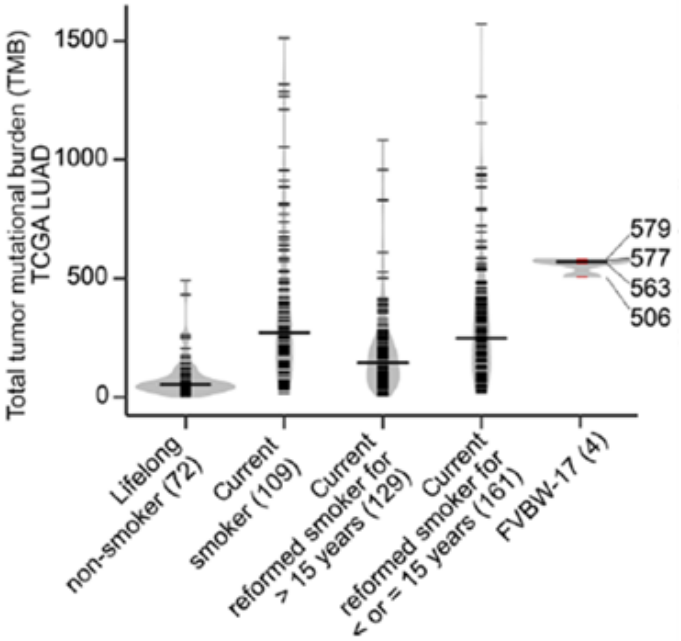
Impact: The FVBW-17 novel cell line is a robust representation of human smoking-related lung adenocarcinoma biology and provides a much-needed preclinical for testing promising new agents and combinations, including immune-based therapies, in an immune-competent environment.

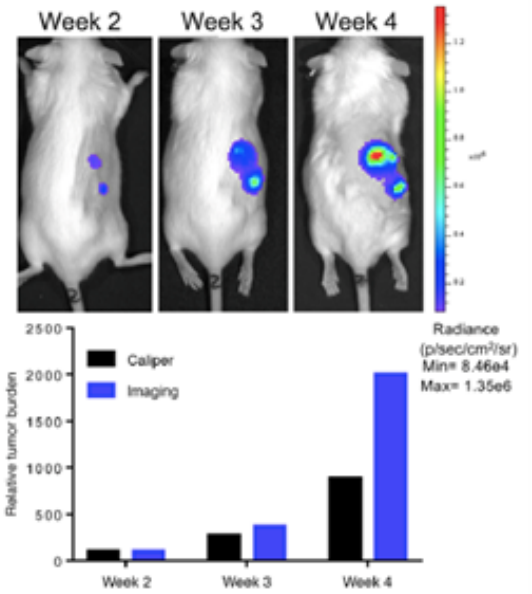
Funding: NCI R01CA204173 (to CJB), A Breath of Hope Foundation Award and American Lung Association Lung Cancer Discovery Award (LCD-257864) (to LPS and TFB), Hillman Fellows for Innovative Cancer Research Program (to LPS and RB), Libby's Lungs (to LPS), and NCI P30CA047904-32.

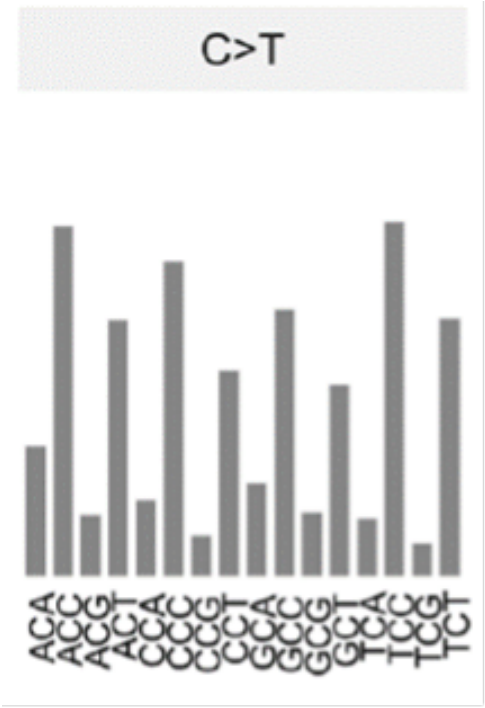
Stabile LP, Kumar V, Gaither-Davis A, Huang EH, Vendetti FP, Devadassan P, Dacic S, Bao R, Steinman RA, Burns TF, Bakkenist BJ. *JCI Insight,* 2021;6(3):e145307. PMID: 33351788

UPMC Hillman Cancer Center Inter-Programmatic Collaboration including:

Drs. Laura P. Stabile, Riyue Bao, Richard A. Steinman, Timothy F. Burns (Cancer Biology), Sanja Dacic (Cancer Therapeutics) and Christopher J. Bakkenist (Genome Stability)



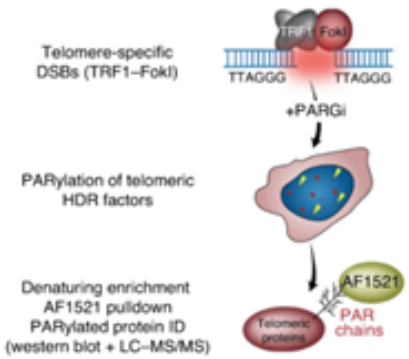


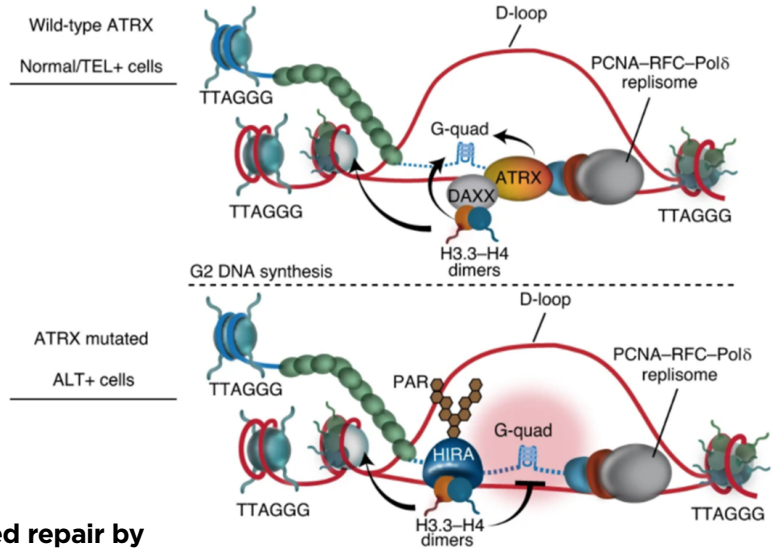


Left: Bioluminescence imaging of lung adenocarcinoma cell line, FVBW-17, tumor growth.

Right: Somatic mutation profile of FVBW-17, represents a classic alkylating signature with validated Kras and Trp53 mutations.

More Cool Science





Regulation of ALT-associated homology-directed repair by polyADP-ribosylation

The synthesis of poly(ADP-ribose) (PAR) reconfigures the local chromatin environment and recruits DNA-repair complexes to damaged chromatin. PAR degradation by poly(ADP-ribose) glycohydrolase (PARG) is essential for the progression and completion of DNA repair. In a study led by Dr. O'Sullivan and his team in collaboration with Dr. Watkins, demonstrated that inhibition of PARG disrupts homology-directed repair (HDR) mechanisms, which underpin alternative lengthening of telomeres (ALT). Proteomic analyses uncover a new role for poly(ADP-ribosyl)ation (PARylation) in regulating the chromatin-assembly factor, HIRA, in ALT cancer cells. They show that HIRA is enriched at telomeres during the G2 phase and is required for histone H3.3 deposition and telomere DNA synthesis. Depletion of HIRA elicits systemic death of ALT cancer cells that is mitigated by re-expression of ATRX, a frequently inactivated protein in ALT tumors. They propose that PARylation enables HIRA to fulfill its essential role in the adaptive response to ATRX deficiency that pervades ALT cancers.

Impact: This proteomic screen discovered an essential protein (HIRA) that is required for cell growth of ALT tumors lacking ATRX and will enable the development of therapeutic options for devastating tumors carrying these mutations

Funding: NCI 5R01CA207209-02 (RJO) and American Cancer Society no. RSG-18-038-01-DMC (RJO); 1S10OD019973-01 (S10); NIH U24CA210967 and R01 GM094231 (AIN); NCI R01CA148629 and NIEHS R01ES014811 (RWS); T32GM008424-25 (NK). This work was also supported by grants C480/A11411 and C5759/A17098 from Cancer Research UK to D.I.J., I.D.W., K.M.S and D.O.; and ERC-2015-ADG-694694

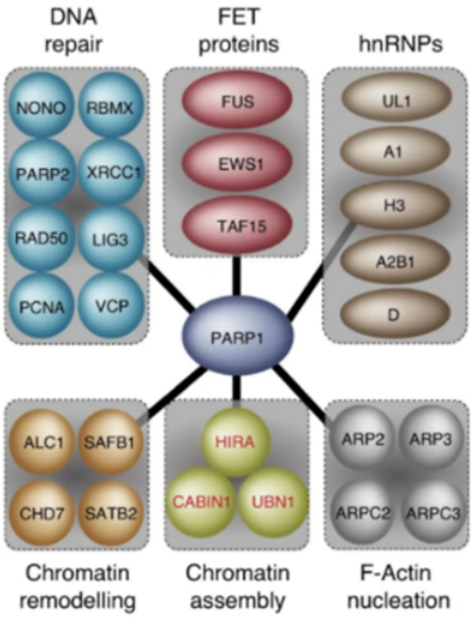
Resource support NCI P30CA047904-32.

Hoang SM, Kaminski N, Bhargava R, Barroso-González J, Lynskey ML, García-Expósito L, Roncaioli JL, Wondisford AR, Wallace CT, Watkins SC, James DI, Waddell ID, Ogilvie D, Smith KM, da Veiga Leprevost F, Mellacharevu D, Nesvizhskii AI, Li J, Ray-Gallet D, Sobol RW, Almouzni G, O'Sullivan RJ.

Nat Struct Mol Biol. 2020 Dec;27(12):1152-1164. PMID: 33046907

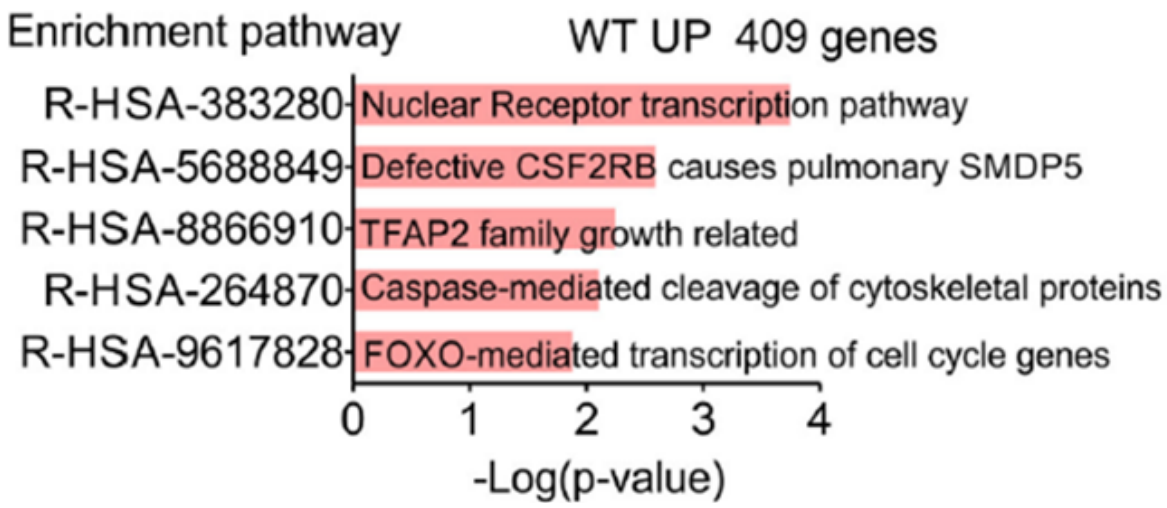
UPMC Hillman Cancer Center Inter-Programmatic Collaboration including:

Drs. Robert E. Schoen (Cancer Epidemiology and Prevention), Xinghua Lu (Cancer Biology), Lin Zhang and Jian Yu (Genome Stability).



Screen for PARylated telomere proteins uncovered several classes of proteins, including the chromatin assembly factor, HIRA. In the absence of ATRX, HIRA working with H3.3 provides telomere maintenance.

More Cool Science



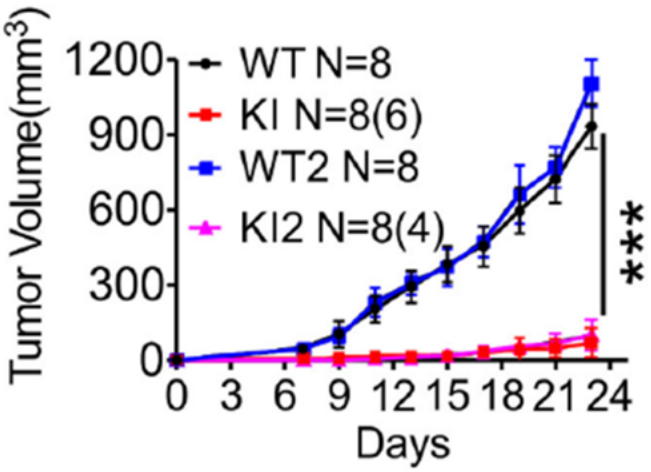
eIF4E S209 phosphorylation licenses myc- and stress-driven oncogenesis.

To better understand the role of eIF4E S209 in oncogenic translation, the Yu and Zhang laboratories working with colleagues, generated EIF4ES209A/+ heterozygous knockin (4EKI) HCT 116 human colorectal cancer (CRC) cells. 4EKI had little impact on total eIF4E levels, cap binding or global translation, but markedly reduced HCT 116 cell growth in spheroids and mice and CRC organoid growth. 4EKI strongly inhibited Myc and ATF4 translation, the integrated stress response (ISR)-dependent glutamine metabolic signature, AKT activation and proliferation in vivo. 4EKI inhibited polyposis in ApcMin/+ mice by suppressing Myc protein and AKT activation. Furthermore, p-eIF4E was highly elevated in CRC precursor lesions in mice and humans. p-eIF4E cooperated with mutant KRAS to promote Myc and ISR-dependent glutamine addiction in various CRC cell lines, characterized by increased cell death,transcriptomic heterogeneity and immune suppression upon deprivation.

Impact: These findings demonstrate a critical role of eIF4E S209-dependent translation in Myc and stress-driven oncogenesis and as a potential therapeutic vulnerability.

Funding: NCI R01CA215481 (JY), R01LM012011 (XL), and NCI R01CA172136 and R01CA203028(LZ).and NCI P30CA047904-32.

Ruan H, Li X, Xu X, Leibowitz BJ, Tong J, Chen L, Ao L, Xing W, Luo J, Yu Y, Schoen RE, Sonenberg N, Lu X, Zhang L, Yu J. *Elife*. 2020 Nov 2;9:e60151. PMID: 33135632



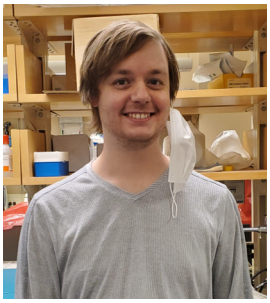
Top: Mutant KRAS and Myc promotes phosphorylation of S209 in the translation elongation factor p-eIF4E, causing up regulation of several critical cellular pathways in response to glutamine, causing glutamine addiction.

Bottom: EIF4ES209A/+ KI colon cancer cells do not support tumor growth.

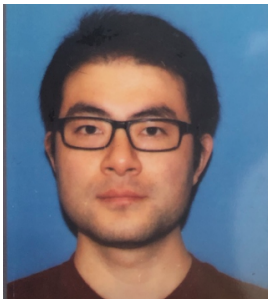
Faculty and Staff News

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

- **Welcome Matthew Schaich, PhD,** Hillman Postdoctoral Fellow for Innovative Cancer Research Program in the Van Houten lab.
- **Welcome Zhou Zhong, PhD,** Postdoctoral fellow in the Van Houten lab.
- **Welcome to Poulomi Nath, PhD,** Postdoctoral fellow in Nechemia-Arbely lab.
- **Welcome Fatimah Adisa,** Research specialist in Opresko lab.
- **Welcome to Amir Salem, PhD,** Visiting scientist from Cairo, Egypt.



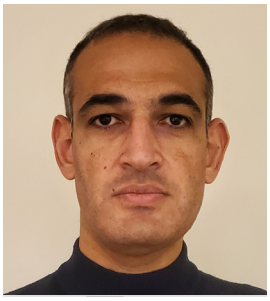
Matthew Schaich



Zhou Zhong

Poulomi Nath

Fatimah Adisa



Amir Salem

Congratulations and best wishes to Sunbok Jang, PhD After an incredibly productive period in the Van Houten laboratory, Dr. Sunbok Jang took a job at the company Hansoh Bio in Rockville, MD on Dec 1, 2020, where he will be putting his outstanding protein purification and structural biology skills to work as a research scientist. Sunbok will be sorely missed, but his legacy of showing that UV-DDB is the first responder to damage during base excision repair (NSMB, 2019) will continue into three additional manuscripts we are preparing for publication.

Van Houten lab celebration with Sunbok's UV-DDB-DNA co-crystal gift on display.

Back row (left to right): Namrata Kumar, Brittani Schnable, Sunbok Jang, and Ben Van Houten

Front row (left to right): Priya Raja, and Rachel Jakielski

Potential Postdoctoral training opportunities at the Hillman Cancer Center:

UPMC Hillman Cancer Center, a National Cancer Institute (NCI)-designated Comprehensive Cancer Center, has launched an exciting new postdoctoral training opportunity at the University of Pittsburgh. Made possible by a donation from the Henry L. Hillman Foundation, the Hillman Postdoctoral Fellows for Innovative Cancer Research program seeks the nation's top graduate students and early-stage postdoctoral fellows to pursue leading-edge cancer research in Hillman laboratories. ***The deadline for application is June 30th, 2021.*** For information and eligibility please see:

Hillman Postdoctoral Fellows for Innovative Cancer Research - UPMC Hillman Cancer Center

Congratulations and best wishes to Braulio Bonilla Villagran, PhD.

Braulio Bonilla Villagran