

DNA Pitt Crew

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



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Note from Director Robert L. Ferris, MD, PhD

During the last six months activities at UPMC Hillman Cancer Center have expanded, despite the continued challenges with COVID-19 and the rise of delta variant infections in our catchment area of western Pennsylvania. As a National Cancer Institute-designated Comprehensive Cancer Center we continue our mission to provide the highest level of clinical care to the nearly 140,000 patients treated at our facilities each year while performing cutting-edge cancer research. To this end we have hired nine new faculty in 2021, including the key strategic recruitments of Taofeek Owonikoko, MD, PhD, and Jeremy Rich, MD, MHS, MBA.

Dr. Owonikoko is chief of the Division of Hematology/Oncology and the Stanley M. Marks - OHA Endowed Chair in the Department of Medicine at the University of Pittsburgh and associate director for Translational Research and co-leader of the Cancer Therapeutics Program at UPMC Hillman Cancer Center. Dr. Owonikoko's research interest includes preclinical biomarker discovery in lung cancer and other solid tumor types, and translation of promising laboratory findings into clinical trials in collaboration with academic and industry partners.

Dr. Rich is the deputy director for research at UPMC Hillman Cancer Center and the Pittsburgh Foundation Chair in Personalized Cancer Therapies and professor in the University of Pittsburgh Department of Neurology. He brings specific expertise into novel therapeutic in the treatment of malignant brain tumors, particularly through a better understanding of their stem cell-like phenotypes which will provide new pharmacologic targets.

Research, the engine of discovery, seeks to uncover novel ways to identify and treat specific tumors, as well as finding the root causes that lead to malignant transformation. As described in the DNA Pitt Crew Newsletter, the Genome Stability Program continues to advance understanding of how genetic alterations can not only cause aggressive tumors but can also provide biomarkers for diagnosis and an opportunity for treatment. We are looking forward to expanding our research enterprise in an adjacent building that is nearing completion.



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

We are very pleased to present the Fall 2021 edition of the DNA Pitt Crew newsletter, which provides recent information about the UPMC Hillman Cancer Center Genome Stability Program (GSP) members, new recruits, and some of the exciting scientific accomplishments from this program. Despite the ongoing challenges that the COVID-19 pandemic continues to present, GSP members with their Hillman collaborators have remained incredibly productive, and we are very proud to share their many accomplishments in recent months. This edition includes four scientific highlights of recently published impactful studies: 1) showing how PARP1 mediates mono- and poly(ADP) ribose addition to itself, proteins, and histones after DNA damage (*Molecular Cell*); 2) defining high-risk molecular profile for distant metastasis in differentiated thyroid cancer (*Cancer*); 3) demonstrating breast cancer cells that are resistant to HSP70 inhibitors upregulate autophagy pathway (*Elife*); and 4) defining how two DNA repair proteins work together at the single molecule level to process 8-oxoG:A mispairs, a potentially mutagenic lesion (*Nucleic Acids Research*). We held a very successful annual GSP mini-retreat virtually, organized by postdoctoral fellow Mariarosaria De Rosa, PhD, and PhD student Kaylee Ermine, during which trainees presented their exciting work. We were especially fortunate to have Graham Walker, PhD, American Cancer Society Professor and Howard Hughes Medical Institute Professor at Massachusetts Institute of Technology and member of the National Academy of Sciences, deliver an engaging keynote lecture complete with historical perspective on his discoveries in the mutagenesis field. We also highlight new grants and awards to GSP members and are especially proud of our F31 PhD student awardees. We wish everyone health and safety as we continue working during this challenging time and look forward to a return to in person meetings.



Inside this issue:

Faculty Spotlight: Dr. Heath Skinner (page 2)
Pitt Stop (page 3)
Conference Highlights and Awards (page 4)
Hot Papers (page 6)
Cool Science (page 6)

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Ben Van Houten, PhD - vanhoutenb@upmc.edu

Faculty Spotlight: Dr. Heath Skinner



Dr. Heath Skinner

Heath D. Skinner, MD, PhD, is an associate professor of radiation oncology and specializes in the study and treatment of head and neck and lung cancer. He is a board-certified radiation oncologist as well as a physician-scientist. His focus remains on providing excellent and compassionate patient care and seeking to integrate clinical trials into clinical practice to improve the lives of his patients.

Dr. Skinner completed a combined MD/PhD program at West Virginia University, followed by a residency in radiation oncology at The University of Texas MD Anderson Cancer Center where he remained on as faculty until joining UPMC Hillman Cancer Center in 2018. Over the course of his career, Dr. Skinner's laboratory has been continuously funded, including three current R01s funding his research. He has published more than 100 peer-reviewed articles in journals such as *Nature Cell Biology*, *Cancer Cell*, *Oncogene*, *Cancer Research*, *Clinical Cancer Research*, and *JAMA Oncology*. In addition, he has written six book chapters and nine invited reviews and presented more than 50 abstracts at national meetings. Dr. Skinner has also been invited to present his work at multiple

domestic and international symposia and serves on the NCI Cancer Biotherapeutics Development Study Section as well as an ad-hoc reviewer on special emphasis panels for PO-1s and SPORES as well as the Cancer Biotherapeutics Development study section. He is overall co-PI for the University of Pittsburgh Head and Neck SPORE, recently submitted for renewal.

Dr. Skinner is a clinical trial safety officer for the National Institute of Dental and Craniofacial Research (NIDCR) and is active in national committees guiding oncologic clinical practice and research, including the NCI HN Steering Committee's Previously Unresected Locally Advanced Task Force and the VA/NIDCR Oropharynx Cancer committee, in addition to being a co-chairman on the NCI TP53 Clinical Trial Planning Committee. His educational track record has been recognized by the American Society of Clinical Oncology, American Society of Radiation Oncology, and the American Board of Radiology, the latter being the accrediting body for radiation oncologists.

Dr. Skinner maintains an active translational research laboratory focused upon identifying novel, clinically targetable biomarkers of resistance to radiation. His group utilizes "big data" approaches to clinical specimens as well as *in vivo* screening techniques to generate novel targets for study. These targets are then further investigated *in vitro* to elicit insights regarding mechanisms of radioresistance. The research in Dr. Skinner's laboratory is designed to generate insights that lead to the rational design of clinical trials using agents that are currently under investigation to minimize the time from bench to bedside. This work led to the completion of two separate clinical trials led by Dr. Skinner evaluating the ability of the common diabetic agent metformin to sensitize tumors to radiation, as well as several additional clinical trials that are either ongoing or close to activation.

Dr. Skinner has four children ranging from two months to eight years old with his lovely wife of 15 years, Lesly Lopez-Skinner. He enjoys spending time with his family, hiking, and running.

Trainee Spotlight: Namrata Kumar



Namrata Kumar

Namrata earned her bachelor's degree in engineering (Biotechnology) from the University of Pune, India. She moved to the United States for a master's degree in Biotechnology at the University of Texas at Dallas. During this time, her research focused on developing an adeno-associated virus (AAV) mediated CRISPR/Cas9 system for *in vitro* and *in vivo* genome editing, which led to a first author publication. Namrata joined Dr. Van Houten's lab as a graduate student researcher in 2017 as a molecular genetics and developmental biology (MGDB) PhD student. As a graduate researcher in the Van Houten lab, Namrata investigates the role of nucleotide excision repair (NER) proteins, UV-DDB, XPA and XPC, in the repair of the oxidative DNA damage, 8-oxoguanine.

Namrata has contributed to two journal articles and five review articles and recently submitted her first-author manuscript to *Nature Communications*. She has received numerous awards including two best poster awards in 2019 and 2020, a best talk award in 2020, the student and new investigator travel award from Environmental Mutagenesis and Genomics Society (EMGS) in 2019 and a Biomedical Graduate Student Association (BGSA) travel award in 2020.

Namrata plans to continue her research in the field of DNA damage response and will be moving to Matthew Weitzman's lab at UPenn for a postdoc.

Pitt Stop: Special Events and Visiting Speakers

GSP Mini-retreat featuring Dr. Graham Walker

June 22, 2021



Dr. Graham Walker



Mariarosaria De Rosa, PhD



Kaylee Ermine

In June, the Genome Stability Program held its annual mini-retreat co-chaired by Mariarosaria De Rosa, PhD, (Opresko Lab) and graduate student Kaylee Ermine (Zhang Lab). The retreat consisted of talks from eight trainees, which are listed below, and concluded with a keynote lecture from distinguished researcher Graham Walker, MD, American Cancer Society Professor, Howard Hughes Medical Institute (HHMI) Professor in the Department of Biology at the Massachusetts Institute of Technology, affiliate member of the Coxe Institute for Integrative Cancer Research and member of the National Academy of Sciences. Dr. Walker was chosen as guest lecturer because of his 45 years of outstanding scientific research activity, in which he studied how cells respond to DNA damage from environmental agents with a particular emphasis on how DNA damage causes mutations and laid the groundwork for the discovery of translesion (TLS) DNA polymerases. Dr. Walker gave an outstanding talk about the “Mutagenic Translesion Synthesis,” guiding the audience through “a Journey from the Ames Test to Cancer Chemotherapy,” and exemplary alternating science and stories about the importance of networking with mentors and trainees for his own personal and scientific growth. In addition

to giving an excellent seminar, Dr. Walker also participated in a virtual Q&A with our GSP trainees, where he was very interested in learning more about each student’s interests and projects. Although virtual, the retreat was well attended and was filled with a lot of exciting science. We are looking forward to next year’s retreat.

2021 GSP Mini-Retreat Trainee Talks:

- Ryan Barnes, PhD (Opresko Lab): Telomeric 8-oxoguanine Drives Premature Senescence Independent of Telomere Shortening
- Thong Luong, graduate student (Bernstein Lab): Regulation of the RECQL4 family during the DNA Damage Response
- Denise Risnik, PhD (Zhang Lab): Chemoimmunotherapy-induced immunogenic cell death in colorectal cancer
- Namrata Kumar, graduate student (Van Houten Lab): Global and transcription-coupled repair of 8-oxoG is initiated by nucleotide excision repair proteins
- Raquel Buj-Gomez, PhD (Aird Lab): The surprising new roles of p16
- Megan Mahlke, PhD (Nechemia-Arbely Lab): Human centromeres drift through cellular proliferation
- Sarah R. Hengel, PhD (Bernstein Lab): RAD51 paralog containing complex SWSAP1-SWS1 stimulates D-loop formation with physiologically relevant substrate RPA coated ssDNA
- Angela Hinchie, Graduate Student (Alder Lab): Telomere dysfunction, independent of length, is sufficient to cause disease in humans

Elise Fouquerel, PhD

Assistant Professor, Department of Biochemistry and Molecular Biology Thomas Jefferson University

July 13, 2021

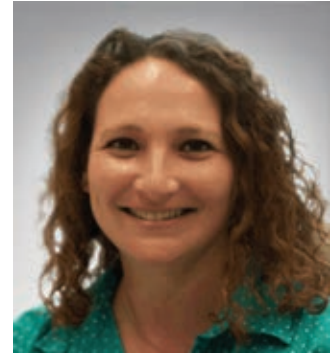
Elise Fouquerel, PhD, visited UPMC Hillman Cancer Center and delivered a hybrid in-person and virtual seminar entitled “Uncovering PARP enzymes and poly(ADP-ribosyl)ation roles in the preservation of telomere integrity.” An alumna of Hillman, Dr. Fouquerel updated us on the remarkable progress she has made since setting up her own research program as an independent PI at TJU. She was recently awarded an NIGMS R35 grant to investigate roles for PARP1 and PARP2 in protecting telomeres and centromeres from oxidative stress induced damage. She described her exciting work investigating PARP1 and PARP2 in base excision repair of oxidative damage at telomeres, and the importance for preventing damage-induced telomere aberrations. She also described interesting new results her lab obtained using a chemoptogenetic tool to selectively induce oxidative base damage at the centromeres. The overall goal of her new research program is to advance understanding of poly(ADP-ribosyl)ation mediated repair in critical genomic regions, and to better understand the impact of PARP inhibitors, which are widely used in cancer therapy, on genome stability. During her visit she met with numerous faculty members at Hillman and across the Pitt campus.



Elise Fouquerel, PhD

Scientific Conference Highlights and Awards

Yael Arbley, PhD: NIH/NIGMS R35 R35GM142717- 01 grant entitled “Mechanisms of epigenetic assembly, maintenance and propagation of human centromeres.” Delivery of chromosomes, the basic units of inheritance, to each daughter cell during cell division is mediated by the centromere. Mammalian centromeres are determined through epigenetic acquisition of a histone H3 variant called CENP-A. This grant will determine the positional stability of human centromeres and whether CENP-A is capable to specify centromere position precisely and stably across a single cell cycle and throughout cellular proliferation at base-pair resolution, using a patient-derived cell line that harbors a neocentromere. The contribution of overexpressed CENP-A and/or HJURP, both known to be elevated in several types of cancer, to human centromere drift and/or expansion will be determined. Next, the relationship between CENP-A binding and DNA methylation at centromeres, neocentromeres, and at ectopic sites of CENP-A deposition will be determined using long-read nanopore sequencing, and the functional importance of centromeric DNA methylation. Using ChIP-sequencing and Cut & Run of CENP-A, CENP-C, and CENP-T/W/S/X nucleosome-like complex at each cell cycle point, the first draft of the CENCODE will be built, an epigenomic landscape of human centromeres, mapped onto the telomere-to-telomere genome assembly, that contains the first description of fully assembled human centromeric genomic maps and DNA methylation data. Finally, innovative single molecule approaches will be used to define histone compositions and combinations of epigenetic posttranslational modifications within single CENP-A-containing nucleosomes located across the genome: at repetitive human centromeres, at non-centromeric ectopic sites, and at neocentromeres.



Yael Arbley

Yi Huang, MD, PhD: New NIH/NCI R01 CA260357-01A1 grant entitled “Role of LSD1 in Triple Negative Breast Cancer Development and Therapeutic Response.” Histone lysine-specific demethylase 1 (LSD1) is a key component of multiple transcription repressor complexes. Tumors in triple negative breast cancer (TNBC) patients frequently express higher level of LSD1 compared to other BC groups. Clinically, LSD1 protein overexpression is significantly associated with worse prognosis in TNBC patients, making it an attractive therapeutic target. However, the mechanism of LSD1 overexpression in TNBC and its role in tumor progression remain to be identified. This grant proposes to identify a novel regulatory mechanism by which overexpression of LSD1 facilitates TNBC progression. This project is also aimed at utilizing novel LSD1-targeting agents to enhance tumor suppression, augment antitumor immunity, and overcome therapeutic resistance in TNBC.



Yi Huang

Karen Arndt, PhD: R35 GM141964 - NIH/NIGMS - “Mechanisms that couple chromatin modifications to transcription.” Proper control of gene expression is fundamentally important for the life and development of all organisms. In all eukaryotes, proteins that regulate transcription, a central step in gene expression, operate within the constraints of a chromatin environment that must be properly managed for gene expression to occur without error. The research program funded by this grant will uncover the functions of the Paf1 complex and other conserved proteins in regulating chromatin structure and transcription—core cellular processes that, when deregulated, cause a multitude of human health conditions, including cancer and developmental defects.



Karen Arndt

Kara Bernstein, PhD: New Department of Defense BC201356 grant entitled “Molecular targeting of homologous recombination-deficient breast cancers”. PARP inhibitors are now FDA approved to treat metastatic breast cancer patients harboring germline *BRCA1* or *BRCA2* mutations. *BRCA1* and *BRCA2* function during DNA repair in the homologous recombination pathway. It has been shown that defects in homologous recombination genes, including *BRCA1/2*, can predict patient response to PARP inhibitors. Importantly there are additional genetic mutations that are found in related homologous recombination genes such as the *RAD51* paralogs, *RAD51C* and *RAD51D*, that are now included on breast/ovarian cancer screening panels. Unfortunately, for individuals with *RAD51C* and *RAD51D* mutations, the vast majority of the mutations uncovered are variants of unknown significance. Therefore, physicians are unable to determine whether these individuals, or their family members, are at increased risk for developing breast cancer or if these patients would benefit from homologous recombination deficient targeted therapies, like PARP inhibitors. This grant is to identify which *RAD51C* and *RAD51D* variants of unknown significance are likely pathogenic by examining their protein interactions, homologous recombination function, and sensitivity to PARP inhibition. Therefore, our work will enable identification of at-risk individuals for breast/ovarian cancer and determine who ultimately will benefit from homologous recombination deficient targeted therapies. 7/1/21-6/30/24.



Kara Bernstein

Angela Hinchie: New NIH/NHLBI F31 HL158063 grant entitled “Discerning the mechanism of telomere dysfunction caused by a mutant telomerase template.” Telomere dysfunction in humans causes a spectrum of clinical phenotypes that affect children and adults. The most common clinical phenotype is pulmonary fibrosis and is thought to be caused by stem cell failure when telomeres become too short. We identified an individual with pulmonary fibrosis that had a variant in the telomere template sequences that caused change in the canonical telomere sequence. This finding provides a one-of-a-kind opportunity to understand the consequences of changes in the telomere sequence in a human family.



Angela Hinchie

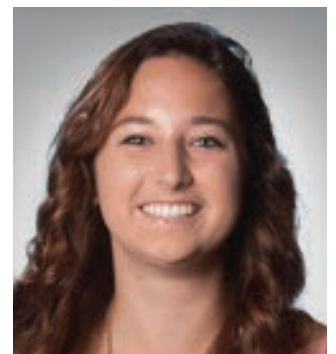
Geyon Garcia, MD/PhD:

R01 ES030335-02S1 - NIH/NIEHS (Diversity Supplement): “Replication fork dynamics and repair by Rad51 paralogues after DNA alkylation.” 3/8/21-5/31/24



Geyon Garcia

Nicole Kaminski: F31 CA264885. Cancer cells ensure replicative immortality through activation of telomere maintenance mechanisms. Approximately 15% of cancers indefinitely lengthen their telomeres through a homology directed repair (HDR) driven mechanism, termed Alternative Lengthening of Telomeres (ALT). This award will fund Nicole’s thesis project which builds on the exciting discovery from the O’Sullivan lab that *RAD51AP1* plays an essential role in telomere maintenance by the ALT pathway. Nicole identified a series of proteins that interact with *RAD51AP1* in cancer cells that activate ALT. She is working to further elucidate the functions of these proteins and understand their functional relationship with *RAD51AP1*.



Nicole Kaminski

Hot Papers

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

L. Yip, W.E. Gooding, A. Nikitski, A.I. Wald, S.E. Carty, E. Karslioglu-French, R.R. Seethala, D.P. Zandberg, R.L. Ferris, M.N. Nikiforova, Y.E. Nikiforov, Risk assessment for distant metastasis in differentiated thyroid cancer using molecular profiling: A matched case-control study, *Cancer*, 127 (2021) 1779-1787.

S. Sannino, M.E. Yates, M.E. Schurdak, S. Oesterreich, A.V. Lee, P. Wipf, J.L. Brodsky, Unique integrated stress response sensors regulate cancer cell susceptibility when Hsp70 activity is compromised, *Elife*, 10 (2021).

A.V. Nikitski, M.N. Nikiforova, L. Yip, E. Karslioglu-French, S.E. Carty, Y.E. Nikiforov, Can TP53-mutant follicular adenoma be a precursor of anaplastic thyroid carcinoma? *Endocr Relat Cancer*, 28 (2021) 621-630.

W.M. Hackeng, L.A.A. Brosens, J.Y. Kim, R. O'Sullivan, Y.N. Sung, T.C. Liu, D. Cao, M. Heayn, J. Brosnan-Cashman, S. An, F.H.M. Morsink, C.M. Heidsma, G.D. Valk, M.R. Vriens, E. Nieveen van Dijkum, G.J.A. Offerhaus, K.M.A. Dreijerink, H. Zeh, A.H. Zureikat, M. Hogg, K. Lee, D. Geller, J.W. Marsh, A. Paniccia, M. Ongchin, J.F. Pingpank, N. Bahary, M. Aijazi, R. Brand, J. Chennat, R. Das, K.E. Fasanella, A. Khalid, K. McGrath, S. Sarkaria, H. Singh, A. Slivka, M. Nalesnik, X. Han, M.N. Nikiforova, R.T. Lawlor, A. Mafficini, B. Rusev, V. Corbo, C. Luchini, S. Bersani, A. Pea, S. Cingarlini, L. Landoni, R. Salvia, M. Milione, M. Milella, A. Scarpa, S.M. Hong, C.M. Heaphy, A.D. Singhi, Non-functional pancreatic neuroendocrine tumours: ATRX/DAXX and alternative lengthening of telomeres (ALT) are prognostically independent from ARX/PDX1 expression and tumour size, *Gut*, (2021).

Cool Science

Unrestrained Poly-ADP-ribosylation Provides Insights into Chromatin Regulation and Human Disease

ADP-ribosylation is a reversible protein post-translational modification that regulates processing and repair of DNA damage. PARP1 mediates mono- and poly-(ADP) ribose (MAR and PAR, respectively) addition to itself, proteins, and histones after DNA damage. In this international collaborative study, Dr. O'Sullivan and colleagues discovered two separate stages of ADP ribosylation whereby PARP1/HPF1 mediated MARYlation at serine residues seeds the PARP1 mediated elongation stage of PARYlation. After DNA damage induced ADP-ribosylation, ARH3 reverses MAR and PARG primarily removes PAR. This team discovered that

Continued

H. Skinner, C. Hu, T. Tsakiridis, R. Santana-Davila, B. Lu, J.J. Erasmus, A.J. Doemer, G.M.M. Videtic, J. Coster, A.X. Yang, R.Y. Lee, M. Werner-Wasik, P.E. Schaner, S.E. McCormack, B.T. Esparaz, R.C. McGarry, J. Bazan, T. Struve, R. Paulus, J.D. Bradley, Addition of Metformin to Concurrent Chemoradiation in Patients With Locally Advanced Non-Small Cell Lung Cancer: The NRG-LU001 Phase 2 Randomized Clinical Trial, *JAMA Oncol*, (2021).

-negative result; addition did not improve survival

E. Prokhorova, T. Agnew, A.R. Wondisford, M. Tellier, N. Kaminski, D. Beijer, J. Holder, J. Gros Lambert, M.J. Suskiewicz, K. Zhu, J.M. Reber, S.C. Krassnig, L. Palazzo, S. Murphy, M.L. Nielsen, A. Mangerich, D. Ahel, J. Baets, R.J. O'Sullivan, I. Ahel, Unrestrained poly-ADP-ribosylation provides insights into chromatin regulation and human disease, *Mol Cell*, 81 (2021) 2640-2655.e2648.

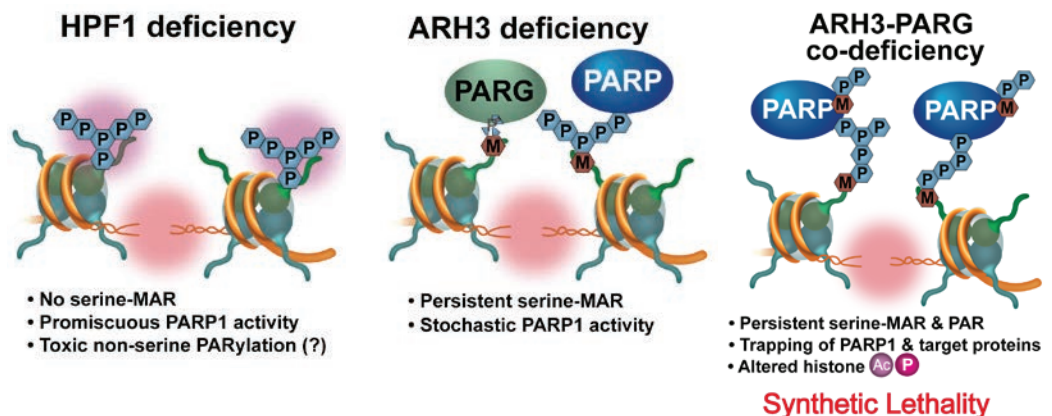
R. Prakash, T. Sandoval, F. Morati, J.A. Zagelbaum, P.X. Lim, T. White, B. Taylor, R. Wang, E.C.B. Desclos, M.R. Sullivan, H.L. Rein, K.A. Bernstein, P.M. Krawczyk, J. Gautier, M. Modesti, F. Vanoli, M. Jasin, Distinct pathways of homologous recombination controlled by the SWS1-SWSAP1-SPIDR complex, *Nat Commun*, 12 (2021) 4255.

Shoshani, S.F. Brunner, R. Yaeger, P. Ly, Y. Nechemia-Arbely, D.H. Kim, R. Fang, G.A. Castillon, M. Yu, J.S.Z. Li, Y. Sun, M.H. Ellisman, B. Ren, P.J. Campbell, D.W. Cleveland, Chromothripsis drives the evolution of gene amplification in cancer, *Nature*, 591 (2021) 137-141.

S. Jang, M.A. Schaich, C. Khuu, B.L. Schnable, C. Majumdar, S.C. Watkins, S.S. David, B. Van Houten, Single molecule analysis indicates stimulation of MUTYH by UV-DDB through enzyme turnover, *Nucleic Acids Res*, 49 (2021) 8177-8188.

Y.P. Yu, S. Liu, J. Nelson, J.H. Luo, Detection of fusion gene transcripts in the blood samples of prostate cancer patients, *Sci Rep*, 11 (2021) 16995.

Figure legend: *HPF1*, histone PARYlation factor 1, deficiency causes *PARP1* to randomly PARYlate chromatin, increasing none serine PARYlation, and loss of serine-MAR, mono-ADP-ribose. *ARH3*, ADP-ribosyl-acceptor hydrolase 3, deficiency causes persistent serine-MAR and increased PAR, poly(ADP)-ribose, accumulation. Finally, deficiency of both *ARH3* and *PARG*, poly(ADP-ribose) glycohydrolase causes persistent serine-MAR and PAR, as well as *PARP1* trapping and altered histone modifications, resulting in synthetic lethality.



More Cool Science

while histone MARYlation persists through the cell cycle and is well tolerated, persistent PARYlation is highly toxic particularly at active histone marks. They found that loss of both ARH3 and PARG is synthetically lethal in human cancer cells, and synergistically suppresses the formation of ALT-associated PML bodies which are involved telomere maintenance in ALT cancers. The synthetic lethality is due to unrestrained ADP ribosylation initiation and elongation steps, causing an excess of toxic PARYlation. However, the loss of ARH3 enables resistance to PARP inhibitors in human cancer cells, including BRCA1/2-deficient cancers.

Impact: PARG inhibitors are being pursued for cancer therapy in combination with DNA damaging agents, and the synthetic

lethality between both PARG and ARH3 inhibition provides a novel therapeutic strategy for killing cancer cells, particular ALT cancers. Furthermore, this study adds ARH3 loss or downregulation to the list of mechanisms by which BRCA1/2-deficient cancers may develop resistance to PARP inhibitor therapy.

Funding: R01CA207209 (R.J.O.) and P30CA047904.

Prokhorova E, Agnew T, Wondisford AR, Tellier M, Kaminski N, Beijer D, Holder J, Gros Lambert J, Suskiewicz MJ, Zhu K, Reber JM, Krassnig SC, Palazzo L, Murphy S, Nielsen ML, Mangerich A, Ahel D, Baets J, O'Sullivan RJ, Ahel I. Mol Cell, 2021. 81: 2640-2655.e2648. PMID: 34019811, PMCID: PMC8221567

Featured Clinical Collaboration: Risk Assessment for Distant Metastasis in Differentiated Thyroid Cancer Using Molecular Profiling: A Matched Case-control Study

Stratifying patients with differentiated thyroid cancer (DTC) is primarily based on determining pathologic characteristics of the tumor. The goal of this outstanding inter-programmatic collaboration led by Dr. Nikiforov with CEP member Dr. Yip and CII members Drs. Ferris and Zandberg, was to characterize the genetic profile of DTC with distant metastasis (DM), to validate molecular-based risk stratification as a method for providing accurate prognosis prior to operation. The team carried out a case-control study for genetic profiling of 62 DTC patients with DM and a matched cohort of DTC patients lacking metastasis after at least five years of follow up by using the ThyroSeq® 3.0 targeted next-generation sequencing assay. Risk for aggressive disease was classified as high, intermediate, and low. Sixty-six percent of DTC patients with DM showed a late-hit mutation in TERT, TP53 or PIK3CA. After propensity matching for age, tumor size and sex, the high-risk molecular profile showed a strong association with DM. Within the 5-10% range of DM observed in DTC, the expected probability of DM would be 0.2-0.4% for the low-risk molecular

profile, 4.7-9.4% for the intermediate-risk molecular profile, and 19.3-33.5% for the high-risk molecular profile. This study revealed that genetic profiling by ThyroSeq 3.0 provides an accurate and robust risk stratification for distant metastasis in patients with differentiated thyroid cancer.

Impact: Over 50,000 new cases of differential thyroid cancer are diagnosed each year in the United States, but most cases will not result in disease-specific mortality. Accurate prognosis by genetic profiling prior to operation may allow for more informed tailoring of treatments for patients with DTC.

Funding: NIH Specialized Programs of Research Excellence (SPORE) grant (P50 CA097190-15).

Yip L, Gooding WE, Nikitski A, Wald AI, Carty SE, Karslioglu-French E, Seethala RR, Zandberg DP, Ferris RL, Nikiforova MN, Nikiforov NE. Cancer, 2021;127(11):1779-1787. PMID: 33539547. PMCID: PMC8113082.

Featured inter-programmatic collaboration:

Jeff Brodsky (Genome Stability Program), Adrian V. Lee, Steffi Oesterreich (Cancer Biology Program), Peter Wipf (Cancer Therapeutics Program) (Genome Stability)

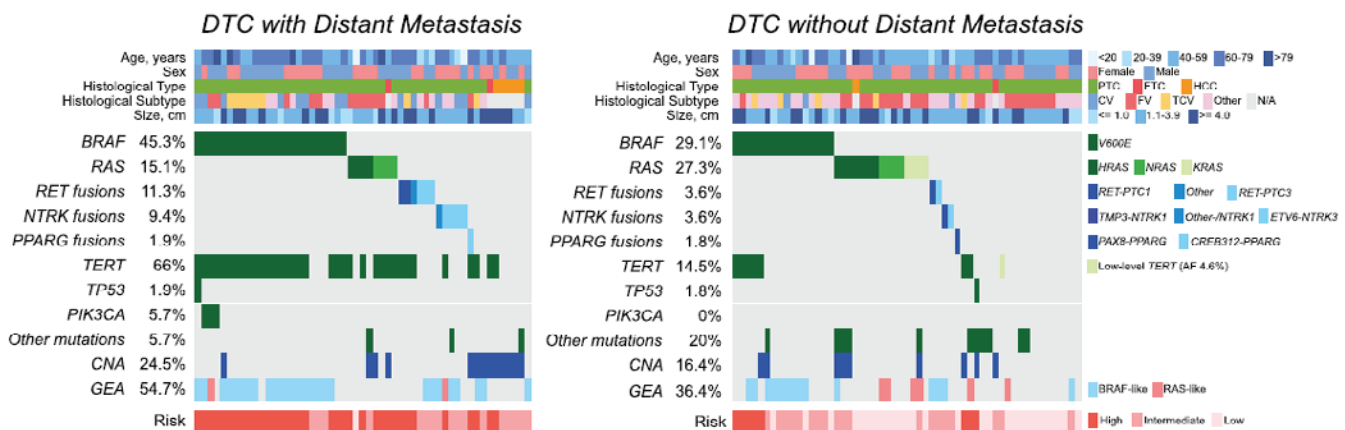


Figure legend: Molecular profiles and features of differentiated thyroid cancer with and without distant metastasis. Copy number alteration (CNA), classic variant (CV), follicular thyroid carcinoma (FTC), follicular variant (FV), gene expression alteration (GEA), Hurthle cell carcinoma (HCC), not applicable (N/A), papillary thyroid carcinoma (PTC), tall cell variant (TCV).

More Cool Science

Featured Inter-programmatic Collaboration:

Jeff Brodsky (Genome Stability Program), Adrian V. Lee, Steffi Oesterreich (Cancer Biology Program), Peter Wipf (Cancer Therapeutics Program)

Unique Integrated Stress Response Sensors Regulate Cancer Cell Susceptibility When Hsp70 Activity is Compromised

DNA damage is known to trigger discrete stress responses, including the unfolded protein response. Molecular chaperones, such as Hsp70, prevent proteotoxicity and maintain homeostasis. This is perhaps most evident in cancer cells, which overexpress Hsp70 and thrive even when harboring high levels of misfolded proteins. To define the response to proteotoxic challenges, this collaborative team of researchers examined adaptive responses in breast cancer cells in the presence of an Hsp70 inhibitor. They discovered that the cells bin into distinct classes based on inhibitor sensitivity. Strikingly, the most resistant cells have higher autophagy levels, and autophagy was maximally activated

only in resistant cells upon Hsp70 inhibition. In turn, resistance to compromised Hsp70 function required the integrated stress response transducer, GCN2, which is commonly associated with amino acid starvation. In contrast, sensitive cells succumbed to Hsp70 inhibition by activating PERK.

Impact: These data reveal an unexpected route through which breast cancer cells adapt to proteotoxic insults and position GCN2 and autophagy as complementary mechanisms to ensure survival when proteostasis is compromised. These insights might provide new methods for treating breast cancer tumors.

Funding: EMBO, ALTF 823-2016, post-doctoral fellowship, SS; NIH [F30CA250167 M.E.Y; R35GM131732, P30DK079307, (J.L.B.) P30CA047904]; HHMI Collaborative Innovation award, (J.L.B.) University of Pittsburgh Translational and Precision, Pharmacology programs (J.L.B.)

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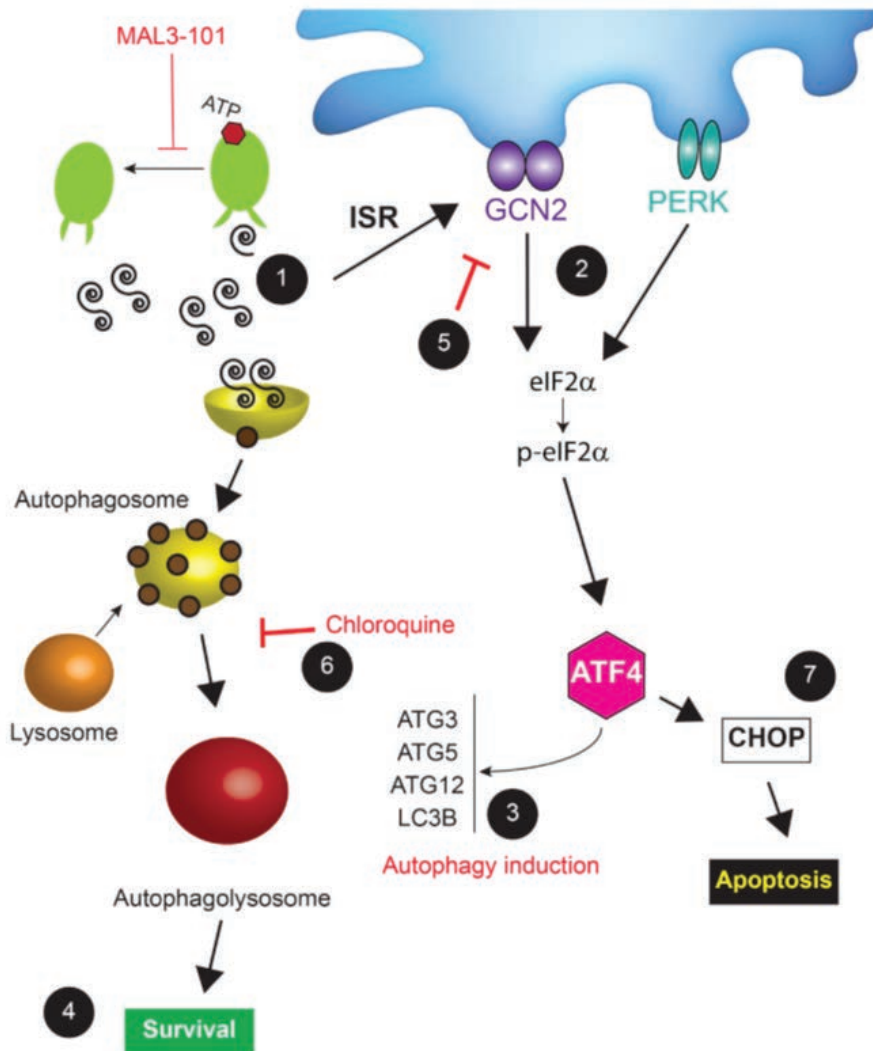


Figure Legend: A model for cancer cell adaptation to Hsp70 inhibition. (1) In MAL3-101 resistant and sensitive cells, Hsp70 inhibition led to activation of the ISR due to the accumulation of misfolded proteins (only misfolded proteins in the cytoplasm are shown). (2) GCN2-mediated eIF2α phosphorylation, which can be induced by amino acid starvation (see Discussion), also induces (3) the transcription of autophagy-related genes, favoring autophagy induction and (4) protecting MAL3-101-resistant breast cancer cells. (5) If GCN2 activity is impaired or (6) autophagy is inhibited (chloroquine), Hsp70 inhibition is cytotoxic so (7) CHOP accumulates and resistant cells undergo apoptosis. (7) In contrast, in MAL3-101-sensitive cells, PERK induces ATF4 and CHOP, which results in cell death.

More Cool Science

Single Molecule Analysis Indicates Stimulation of MUTYH by UV-DDB through Enzyme Turnover

The oxidative base damage, 8-oxo-7,8-dihydroguanine (8-oxoG) is a highly mutagenic lesion because replicative DNA polymerases insert adenine (A) opposite 8-oxoG. In mammalian cells, the removal of A incorporated across from 8-oxoG is mediated by the glycosylase MUTYH during base excision repair (BER). Mutations in this protein are associated with increased colon cancer. After A excision, MUTYH binds avidly to the abasic site and is thus product inhibited. The Van Houten group has previously reported that UV-DDB plays a non-canonical role in BER during the removal of 8-oxoG by 8-oxoG glycosylase, OGG1 and presented preliminary data that UV-DDB can also increase MUTYH activity. In this present study this inter-institutional team examined the mechanism of how UV-DDB stimulates MUTYH. Bulk kinetic assays show that UV-DDB can stimulate the turnover rate of MUTYH excision of A across from 8-oxoG by four- to five-fold. Electrophoretic mobility shift assays and atomic force microscopy suggest transient complex formation between MUTYH and UV-DDB, which displaces MUTYH from abasic sites. Using single molecule

fluorescence analysis of MUTYH bound to abasic sites, we show that UV-DDB interacts directly with MUTYH and increases the mobility and dissociation rate of MUTYH. UV-DDB decreases MUTYH half-life on abasic sites in DNA from 8800 to 590 seconds. Together these data suggest that UV-DDB facilitates productive turnover of MUTYH at abasic sites during 8-oxoG:A repair.

Impact: Mice deficient in DDB2, the DNA damage recognition subunit of UV-DDB die prematurely due to spontaneous solid tumors. These data combined with this groups previous results suggest that UV-DDB helps to recognize spontaneous DNA damage, which can drive mutations and cancer.

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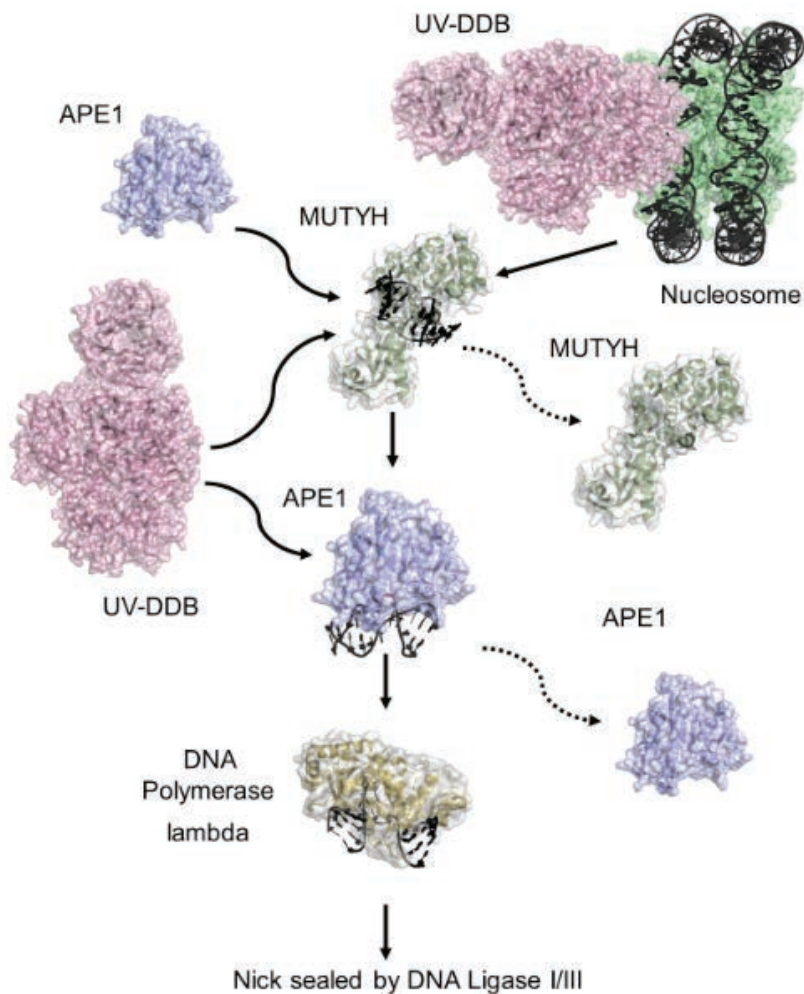


Figure Legend: Proposed working model of UV-DDB stimulation of MUTYH in the repair of 8-oxoG. (A) Schematic representation of the proposed BER pathway including UV-DDB is illustrated. UV-DDB is believed to be rapidly recruited to damaged sites in chromatin and help facilitate processing by MUTYH. Biochemical and single molecule data suggest that UV-DDB transiently associates with MUTYH at 8-oxoG:abasic sites to increase its release and turnover. APE1 is expected to be necessary to incise the DNA and has been shown previously to be stimulated by UV-DDB. DNA pol λ then undergoes long patch repair (3), and FEN1 (not shown) processes the flap leaving a nick, which is then sealed by DNA ligase I/III.

Faculty and Staff News



Jacob Stewart-Ornstein, PhD.

Farewell to Dr. Stewart-Ornstein

Congratulations and best wishes to Dr. Stewart-Ornstein who has accepted the position of Assistant Director of Off Target Biology at Prime Medicine in Cambridge, Massachusetts. Prime Medicine uses “Prime Editing”, a next-generation technology that can “search and replace” to restore normal genetic function almost anywhere in the genome. Prime Medicine was founded to bring the promise of gene editing to patients.

Farewell, Dr. Zhou Zhong

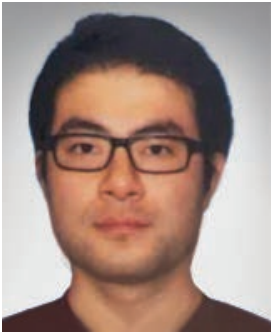
Best of luck to Zhou Zhong, PhD, in his new endeavors.

We are pleased to welcome the following new faculty members:

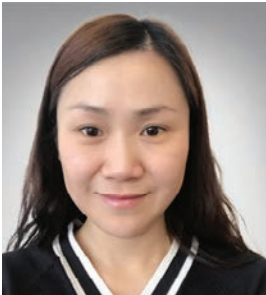
Wei Du, PhD, joined the Genome Stability Program and is an associate professor in the Department of Medicine.

Dr. Kara Bernstein’s lab welcomed the following newcomers:

- **Adeola Fagunloye**, graduate student in Pharmacology and Chemical Biology
- **Geyon Garcia, MD/PhD** student in Molecular Genetics and Developmental Biology
- **Christie Darrah**, postdoctoral associate



Zhou Zhong



Wei Du



Adeola Fagunloye



Geyon Garcia



Christie Darrah