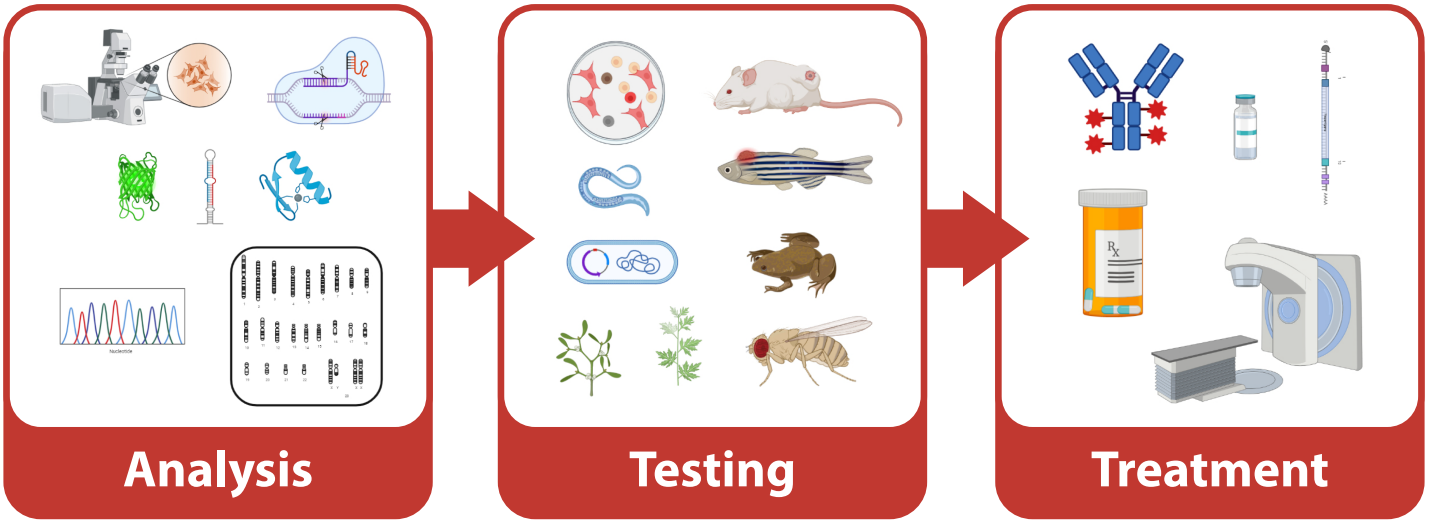
NIH NATIONAL CANCER INSTITUTE

The Center for Cancer Research Fellows and Young Investigators Steering Committee presents:

21st Annual CCR-FYI COLLOQUIUM

From Mechanisms to Therapies: Current Highlights in Cancer Research



April 19–20, 2021

Program

U.S. Department of Health & Human Services | National Institutes of Health

21st Annual Center for Cancer Research Fellows and Young Investigators (CCR-FYI) Colloquium

SCHEDULE AND PROGRAM BOOK

From Mechanisms to Therapies: Current Highlights in Cancer Research

April 19th- 20th, 2021

Welcome Letter

Welcome Colloquium Participants!

On behalf of the NCI Center for Cancer Research Fellows and Young Investigators (CCR-FYI) Steering Committee and CCR-FYI Colloquium Subcommittee, we welcome you to the 21st Annual CCR-FYI Colloquium. The CCR-FYI strives to promote scientific, career, and personal success and growth among all postdoctoral fellows, clinical fellows, postbaccalaureate fellows, and graduate students on the NIH campuses. We are kindly assisted by the NCI's Center for Cancer Research (CCR) Office of the Director and the Center for Cancer Training (CCT) Office of Training and Education, who work to enhance the intramural trainee experience. The CCR-FYI would like to thank Dr. Ned Sharpless, Dr. Glenn Merlino, Dr. William Dahut, Dr. Tom Misteli, Erika Ginsburg, Dr. Oliver Bogler for their continuing assistance and guidance. We would also like to thank Angela Jones from the Center for Cancer Training, Robert Montano and his team from the Center for Biomedical Informatics and Information Technology for providing us with the managerial and technical help, respectively. To achieve our mission of promoting CCR scientists coming together to share ideas, foster collaborations, gain knowledge and increase skills needed to attain their career goals, the CCR-FYI organizes opportunities including the Annual CCR-FYI Colloquium, the CCR-FYI seminar series, networking and outreach events, and the CCR-FYI Newsletter.

In celebrating the 21st Annual CCR-FYI Colloquium, we look back at the year 2020, the year of the Covid19 pandemic, and the year when we saw the cancellation of our annual Colloquium 2020, only to strive forward to organize the first ever virtual one-day CCR-FYI Symposium. This year, based on the existing Covid19 prevention guidelines and considering the health and safety of our NCI colleagues, we decided to host a two-day virtual Colloquium. The theme for the Colloquium, "From Mechanisms to Therapies: Current Highlights in Cancer Research", explores various stages of therapeutic development and translating research from lab to clinic. We hope the NCI community will take this opportunity to share exciting discoveries, network with their peers, and fuel their passion for our overarching goal of eliminating cancer. This year's Colloquium will feature three workshops aiming to guide fellows through the application and interview process for either an academic or non-academic position as well as a workshop focused on how to improvise management techniques.

CCR-FYI Colloquium 2021 has brought together keynote speakers who are leading experts in the fields of cancer and human immunodeficiency virus (HIV). Dr. J. Carl Barrett, Vice President at AstraZeneca, Oncology Translational Sciences, the founding director of the CCR; will discuss about his innovative translational research on cancer biomarkers and epigenetic changes of cancer cells. Dr. Alexandra Newton from the University of California, San Diego, will talk about her exceptional work in the regulation of tumor suppressor enzymes. Dr. Michael Gottesman, Deputy Director of Intramural Research, Chief of the Laboratory of Cell Biology, will talk about his research on identifying the various mechanisms of drug-resistance in cancer cells and how to therapeutically target these vulnerabilities. Dr. Barbara Felber, Senior Investigator of the Vaccine Branch at NIH, Head of the Human Retrovirus Pathogenesis Section, will discuss about the current highlights on the use of DNA-based vaccine strategies both as preventive and immunotherapeutic approaches to control HIV. We will also have a keynote talk from our Outstanding Postdoctoral Fellow awardee for 2021, Dr. Sachi Horibata, Postdoctoral Research Fellow, Laboratory of Cell Biology, CCR, NCI who will present her work on understanding the mechanism of chemotherapy resistance in acute myeloid leukemia and in ovarian cancer. There will be also be an inspiring cancer survivorship presentation by Dr. Marty Tenenbaum who is a cancer survivor, patient advocate, computer scientist and the Founder of Cancer Commons and CollabRx.

The 21st Annual Colloquium provides excellent opportunities to learn about your colleagues' research, advance your scientific knowledge, establish new collaborations, prepare for the next stage of your career, and network. We hope that you capitalize on as many of these opportunities as possible and enjoy this year's Colloquium.

Thank you for your participation this year,

CCR-FYI Colloquium Subcommittee Co-chairs:
Srikanta Basu, Ph.D. and Katelyn Ludwig, Ph.D.
& the CCR-FYI Colloquium Planning Committee

Monday, April 19, 2021

8:30 – 8:45 a.m. **Opening Remarks from Colloquium Planning Committee Chairs: Srikanta Basu, Ph.D. and Katelyn Ludwig, Ph.D.**

8:45 – 9:00 a.m. **Remarks from Center for Cancer Training Leadership**
Erika Ginsburg, M.A., Chief, Office of Training and Education, CCT
Oliver Bogler, Ph.D., Director, Center for Cancer Training

9:00 – 9:30 a.m. **Ned Sharpless, M.D.,** Director, NCI

9:30 – 10:30 a.m. **Keynote Speaker I**
Michael Gottesman, M.D., NIH Deputy Director for Intramural Research & Chief of the Laboratory of Cell Biology
"The Role of Multidrug Transporters in Drug Resistance in Cancer"

10:30 – 10:45 a.m. **BREAK**

10:45 – 12:00 p.m. **Concurrent Oral Presentations (3 virtual breakout sessions)**

a) **Cancer Models, Cancer Stem Cells, Carcinogenesis and Metastasis**
- Arun Prakash Mishra, Ph.D.
- Dan Li, Ph.D.
- Woo Yong Park, Ph.D.
- Dimitris Stellas, Ph.D.

b) **Genetics, Genomics, Chromatin, Signal Transduction, and Transcription**
- Michael Boylan, Ph.D.
- Vernon Ebegboni, Ph.D.
- Arun Kumar Ganesan, Ph.D.
- Konnie Guo

c) **Immunology, Virology, and Metabolism**
- Aarti Kolluri
- Tristan Neal
- Mary L. Piaskowski
- Madeline Spetz

12:00 – 12:30 p.m. **LUNCH**

12:30 – 2:00 p.m. **Virtual Poster Session I**

a) Bioinformatics, Epidemiology, and Translational Research
- Rahulsimham Vegesna, Ph.D.

b) Molecular and Cellular Biology and Microbiology
- Dalen Chan, Ph.D.
- Samuel Chen
- Molly D. Congdon, Ph.D.
- Garis Grant

- Mariana D. Mandler, Ph.D.
- Zhilin Yang, Ph.D.

c) Genetics, Genomics, Chromatin, Signal Transduction and Transcription

- Sitanshu Singh, Ph.D.
- Soumya Sundara Rajan, Ph.D.

d) Immunology, Virology, and Metabolism

- Chad H. Hogan
- Sevasti Karaliota, Ph.D.
- Neha Wali

2:00 – 2:15 p.m. **BREAK**

2:15 – 3:15 p.m. **Keynote Speaker II**

Alexandra Newton, Ph.D., Distinguished Professor of Pharmacology at the University of California, San Diego
"Reversing the Paradigm: Protein Kinase C as Tumor Suppressive"

3:15 – 4:00 p.m. **Outstanding Postdoctoral Fellow**

Sachi Horibata, Ph.D., Postdoctoral Research Fellow, Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute
"Identifying underlying determinants of chemotherapy resistance in cancer"

4:00 – 4:30 p.m. **BREAK**

4:30 – 5:45 p.m. **Concurrent Workshops and Panels I (3 virtual breakout sessions)**

I. **Management Techniques** *(Workshop)*
Shannon Bell, MSW, Director of the NCI Office of Workforce Planning and Development

II. **Academia** *(Panel)*
Kirill Afonin, Ph.D., Associate Professor, Department of Chemistry, UNC-Charlotte
Emma Benn, DrPH, Associate Professor, Center for Biostatistics and Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai
Alexandra Newton, Ph.D., Professor, Department of Pharmacology, UC-San Diego
Regina Nuzzo, Ph.D., Professor, Department of Science, Technology & Mathematics, Gallaudet University

III. **Non-Profit Organizations** *(Panel)*
Lynn Marquis, B.A., Director at the Coalition for the Life Sciences
Laurel Oldach, Ph.D., Scientific Communicator at the American Society for Biochemistry and Molecular Biology
Erin Rosenbaugh, Ph.D., P.M.P., Associate Scientific Project Manager for Neuroscience at the Foundation for the National Institutes of Health
Richard Turman, M.P.P., President at ACT for NIH

Tuesday, April 20, 2021

8:30 – 8:45 a.m. **CCR Basic Science Director's Address**
Glenn Merlino, Ph.D., CCR Scientific Director for Basic Research

8:45 – 9:00 a.m. **CCR Clinical Director's Address**
William L. Dahut, M.D. CCR Scientific Director for Clinical Research, NCI Clinical Director

9:00 – 10:00 a.m. **Keynote Speaker III**
Dr. Barbara Felber, Ph.D., Senior Investigator of Vaccine Branch at NIH, and Head of the Human Retrovirus Pathogenesis Section
"DNA vaccine for broad and durable immunity"

10:00 – 10:15 a.m. **BREAK**

10:15 – 11:30 a.m. **Concurrent Oral Presentations II (3 virtual breakout sessions)**

a) Bioinformatics, Epidemiology, and Translational Research
- Nishanth Ulhas Nair, Ph.D.
- Welles Robinson
- Fiorella Schischlik, Ph.D.
- Pedro Torres-Ayuso, Ph.D.

b) Biophysics, Chemistry, Pharmacology and Structural Biology
- Sumirtha Balaratnam, Ph.D.
- Sophia A. Lookingbill
- Woong Young So, Ph.D.
- Tam Vo, Ph.D.

c) Molecular and Cellular Biology and Microbiology
- Christina M. Fitzsimmons, Ph.D.
- T. Lily Nguyen
- Debasish Paul, Ph.D.
- Gabrielle C. Puller

11:30 – 12:00 p.m. **LUNCH**

12:00 – 1:30 p.m. **Virtual Poster Sessions II**

a) Bioinformatics, Epidemiology, and Translational Research
- Rahulsimham Vegesna, Ph.D.

b) Molecular and Cellular Biology and Microbiology
- Dalen Chan, Ph.D.
- Samuel Chen
- Molly D. Congdon, Ph.D.
- Garis Grant
- Mariana D. Mandler, Ph.D.
- Zhilin Yang, Ph.D.

c) Genetics, Genomics, Chromatin, Signal Transduction and Transcription

- Sitanshu Singh, Ph.D.
- Soumya Sundara Rajan, Ph.D.

d) Immunology, Virology, and Metabolism

- Chad H. Hogan
- Sevasti Karaliota, Ph.D.
- Neha Wali

1:30 – 1:45 p.m. **BREAK**

1:45 – 2:45 p.m. **Keynote Speaker IV**

J. Carl Barrett, Ph.D., Vice President at AstraZeneca, Oncology Translational Sciences, Founding Director of CCR, and Keynote Speaker at Inaugural Colloquium

"Translational Medicine in Driving Drug Development in Oncology"

2:45 – 3:30 p.m. **Survivorship Speaker**

Marty Tenenbaum, Ph.D., Computer Scientist and Cancer Survivor, Founder of Cancer Commons and CollabRx

3:30 – 3:45 p.m. **BREAK**

3:45 – 5:00 p.m. **Concurrent Workshops and Panels II (3 virtual breakout sessions)**

I. **Virtual Interview Techniques & Interview Skills** *(Workshop)*
Scott Morgan, M.A., Senior Associate, Center for Strategic and International Studies

II. **Networking and Scientific Communication** *(Workshop)*
Phil Ryan, Ph.D., Deputy Director of Graduate Programs and Student Services, NIH Office of Intramural Training & Education

III. **Drug Development and Industry** *(Panel)*
J. Carl Barrett, Ph.D., Vice President & Global Head of Translational Medicine, Oncology R&D, AstraZeneca
Scott Martin, Ph.D., Director, Functional Genomics & Principal Scientific Manager, Department of Discovery Oncology, Genentech
Nicole Schiavone, Ph.D., Senior Scientist, Pfizer
Matthew Meyer, Ph.D., Senior Director and Head, Discovery Pharmacology and In Vivo Biology, Bristol Myers Squibb

5:00 – 5:45 p.m. **Closing Address & Travel Awards**

Tom Misteli, Ph.D., Director, Center for Cancer Research

Leadership

Amy Funk, Ph.D.
CCR-FYI Co-Chair

Dr. Amy L. Funk received her Ph.D. in Chemistry in the laboratory of Dr. Christine Hrycyna at Purdue University in West Lafayette, IN in 2017. She studied a membrane protein implicated in K-Ras driven cancers, specifically pancreatic cancer. She focused on elucidating the substrate binding site within the protein to aid in the design and development of inhibitors. Dr. Funk is currently a postdoctoral fellow in the laboratory of Dr. John Brognard in the Laboratory of Cell and Developmental Signaling at NCI-Frederick. She is defining the role of the understudied leucine zipper-bearing kinase (LZK) as a novel therapeutic target in head and neck squamous cell carcinoma patients and improving current treatments through small molecule inhibitors and protein degradation utilizing PROTAC technology.

Jessica Eisenstatt, Ph.D.
CCR-FYI Co-Chair

Dr. Jessica R. Eisenstatt is currently an Interagency Oncology Task Force fellow with the NCI and FDA. In this position, she performs regulatory research and review under the mentorship of Dr. Deborah A. Hursh. Her research focuses on the genomic instability of induced pluripotent stem cells. Dr. Eisenstatt received her Ph.D. in Biochemistry in the laboratory of Dr. Kurt W. Runge at Case Western Reserve University in Cleveland, Ohio in 2016 where she studied the role of histone modifications in the DNA damage response and telomere maintenance in fission yeast. She did her postdoctoral fellowship in the lab of Dr. Munira A. Basrai of the Genetics Branch at NCI-Bethesda. Her research in Dr. Basrai's lab focused on determining molecular mechanisms that regulate the localization pattern of centromere-specific proteins to promote faithful chromosome segregation in budding yeast.

NCI Leadership

Erika Ginsburg, M.A.
Chief of the Office of Training and Education
Center for Cancer Training
National Cancer Institute

Erika currently serves as Chief of NCI's Center for Cancer Training (CCT) Office of Training and Education (OTE). She uses her passion for training and mentorship to manage career development programs for trainees. Several years ago, Erika revised the Sallie Rosen Kaplan Postdoctoral Fellowship for Women Scientists to better prepare NCI's female postdoctoral fellows to transition to independence and to retain them in science. Fellows who have successfully completed the one-year program and have gone on to their next career stage have all remained in the biomedical workforce. In addition, she has developed, coordinated, and evaluated other career development workshops, courses, and programs in the CCT. Erika leads the NIH Fellows Editorial Board, NCI Explore On-Site program, and the NCI Director's Innovation Award, just to name a few. Her effort in initiating the Responsible Conduct of Research training course for NCI trainees was recognized by an individual NCI's Director's Award in 2016. In 2006, the Association for Women in Science, Bethesda Chapter, awarded her Mentor of the Year. As Chief of OTE, Erika will further advocate for trainees, and continue to facilitate and promote training opportunities by working closely with trainees, PIs, and senior leadership.

Before joining CCT, Erika had a long career in NCI's intramural program as a Technical Laboratory Manager in CCR's Mammary Biology and Tumorigenesis Laboratory where she studied prolactin's action on breast cancer. Prolactin is an important hormone responsible for the development of the breast and may be positively associated with breast cancer risk. Together with Dr. Barbara Vonderhaar, she was the first to demonstrate that human breast cancer cells synthesize and secrete significant amounts of biologically active prolactin. She mentored over 150 trainees in the laboratory and has over 50 publications in the fields of drug metabolism, and hormone regulation of the normal and cancerous breast.

Erika received her undergraduate training in Biophysics and Microbiology from the University of Pittsburgh, her master's degree from Johns Hopkins University in Science/Medical Writing, and holds a master's Certificate in Biotechnology Management from the University of Maryland University College.

Oliver Bogler, Ph.D.
Director of the Center for Cancer Training
National Cancer Institute

Oliver studied Natural Sciences at Cambridge University, completed his PhD at the Ludwig Institute for Cancer Research in London, and post-doctoral training at the Salk Institute and the Ludwig Institute, San Diego Branch. He has held faculty appointments at Virginia Commonwealth University, Henry Ford Hospital and the University of Texas MD Anderson Cancer Center where he also served as Director of Basic Research for the Brain Tumor Center. His research focused on EGFR signaling and novel platinum compounds in glioblastoma.

In 2010, he became MD Anderson's Vice President for Global Academic Programs and managed a network of 35 Sister Institutions in 22 countries, with a total investment in global cancer collaborations of $24M over 7 years and an annual conference with over 800 participants. In 2011, he was also appointed Senior Vice President for Academic Affairs where he stewarded MD Anderson's education mission including career development and accreditation, and oversaw 300 people, who delivered support for 1,700 faculty and more than 2,000 trainees and students.

Dr. Bogler joined the ECHO Institute in 2018 as Chief Operating Officer to support its efforts to democratize scarce expert knowledge to improve services to the underserved in healthcare, education and beyond, including in cancer.

In 2020 Oliver joined NCI as the Director of the Center for Cancer Training, where he focuses on NCI training and career development programs that catalyze the development of a 21st century workforce capable of advancing cancer research through a scientifically integrated approach.

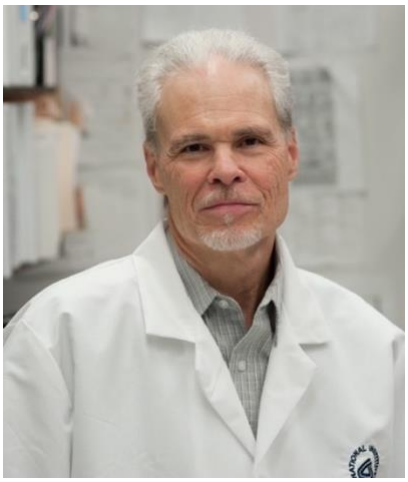
Ned Sharpless, M.D.
Director of the National Cancer Institute
National Institutes of Health

Norman E. “Ned” Sharpless, M.D., was officially sworn in as the 15th director of the National Cancer Institute (NCI) on October 17, 2017. Prior to his appointment, Dr. Sharpless served as the director of the Lineberger (“line burger”) Comprehensive Cancer Center at the University of North Carolina (UNC).

Dr. Sharpless was a Morehead Scholar at UNC–Chapel Hill and received his undergraduate degree in mathematics. He went on to pursue his medical degree from the UNC School of Medicine, graduating with honors and distinction in 1993. He then completed his internal medicine residency at the Massachusetts General Hospital and a hematology/oncology fellowship at Dana-Farber/Partners Cancer Care, both of Harvard Medical School in Boston. After 2 years on the faculty at Harvard Medical School, he joined the faculty of the UNC School of Medicine in the Departments of Medicine and Genetics in 2002. He became the Wellcome Professor of Cancer Research at UNC in 2012.

Dr. Sharpless is a member of the Association of American Physicians and the American Society for Clinical Investigation and is a Fellow of the Academy of the American Association of Cancer Research. He has authored more than 160 original scientific papers, reviews, and book chapters, and is an inventor on 10 patents. He cofounded two clinical-stage biotechnology companies: G1 Therapeutics and Sapere Bio (formerly HealthSpan Diagnostics). He served as Acting Commissioner for Food and Drugs at the US FDA for seven months in 2019, before returning to the NCI Directorship.

Glenn Merlino, Ph.D.
CCR Scientific Director for Basic Research
National Cancer Institute



Dr. Glenn Merlino's career research contributions include the areas of receptor tyrosine kinase signaling, oncogenic transformation, transcriptional regulation, cell cycle regulation, multiple drug resistance, genomic instability, and genetically engineering mice to generate models to study human cancer. Dr. Merlino was the first to report the amplification/rearrangement of the Epidermal Growth Factor Receptor gene in human cancer and was among the first to show that growth factors could function in vivo as oncogenes. Dr. Merlino and his colleagues are seeking to elucidate the complex molecular/genetic programs governing melanoma genesis and progression through development and analysis of genetically engineered mouse models. Using such models Dr. Merlino provided the first experimental evidence supporting the notion that childhood sunburn is a critical melanoma risk factor. His aim is to continue to uncover mechanisms associated with UV-mediated induction of melanoma and its progression to the metastatic state. Currently, a particular emphasis is on modeling melanoma responses to clinically relevant pathway-targeted and immune-based therapies. Preclinical models have now been developed that are anticipated to help uncover mechanisms by which melanomas survive and develop resistance to immune checkpoint inhibitors, in order to devise improved clinical strategies to more effectively treat patient melanomas and prevent their recurrence.

William L. Dahut, M.D.
CCR Scientific Director for Clinical Research
National Cancer Institute

Dr. Dahut received his M.D. from Georgetown University in Washington, DC. He completed clinical training in internal medicine at the National Naval Medical Center in Bethesda, MD, followed by training in hematology and medical oncology at the Bethesda Naval Hospital and the Medicine Branch of the NCI. Dr. Dahut worked as an attending physician in the NCI-Navy Medical Oncology Branch until 1995. He then joined the faculty of the Lombardi Cancer Center at Georgetown University before returning to NCI in 1998. Dr. Dahut's primary research interest has been in the development of novel therapeutic strategies for the treatment of adenocarcinoma of the prostate. He has pioneered the effort to combine experimental therapies such as angiogenesis and immunotherapy with chemotherapy, androgen blockade, radiotherapy and other more traditional modalities. His recent studies combining immunotherapy with more standard treatment have been active and demonstrated the ability to maintain a robust immunologic response.

In 2009, Dr. Dahut was appointed as CCR Clinical Director where he oversees and assures the quality of medical care delivered to patients participating in CCR clinical trials. In 2012, Dr. Dahut was appointed as a CCR Deputy Director then in 2016 was selected to become the CCR Scientific Director for Clinical Research.

Tom Misteli, Ph.D.
Director of the Center for Cancer Research
National Cancer Institute

Tom Misteli is an NIH Distinguished Investigator and the Director of the Center for Cancer Research at the National Cancer Institute, NIH, overseeing the basic and clinical activities of more than 250 NCI Principal Investigators. He is an internationally renowned cell biologist who pioneered the use of imaging approaches to study the 3D organization of genomes and gene expression in living cells. His laboratory's interest is to uncover the fundamental principles of genome architecture and function and to apply this knowledge to the development of novel diagnostic and therapeutic strategies for cancer and aging. He obtained his PHD from the University of London, UK and performed post-doctoral training at the Cold Spring Harbor Laboratory. For his work he has received numerous awards including the Mendel Medal, the Herman Beerman Award, the Wilhelm Bernhard Medal, the Gold Medal of the Charles University, the Flemming Award, the Gian-Tondury Prize, the NIH Director's Award.

He is an elected Fellow of the American Society for Cell Biology. He acts as an advisor for numerous national and international agencies and serves on several editorial boards including *Cell, Science* and *PLoS Biology.* He is the Editor-in-Chief of Current *Opinion in Cell Biology.*

Keynote Speakers

Michael Gottesman, M.D.
NIH Deputy Director for Intramural Research &
Chief of the Laboratory of Cell Biology, National Cancer Institute

Michael Gottesman has been Deputy Director for Intramural Research at NIH since 1993. A graduate of Harvard College and Harvard Medical School, Dr. Gottesman completed an internship and residency at the Peter Bent Brigham Hospital in Boston and was a research associate at NIH from 1971 to 1974. He returned to Harvard Medical School as an assistant professor in the Department of Anatomy before returning to NIH in 1976. Michael became Chief of the Laboratory of Cell Biology in the National Cancer Institute in 1990. From 1992 to 1993, he was Acting Director of the National Center for Human Genome Research, and he was Acting Scientific Director of the NCHGR in 1993. He is an elected fellow of the AAAS and the American Association of Physicians and has been a member of the National Academy of Medicine since 2003, the American Academy of Arts and Sciences since 2008, and the National Academy of Sciences since 2018.

Michael's research interests have ranged from how DNA is replicated in bacteria to how cancer cells elude chemotherapy with over 500 peer-reviewed scientific publications. His laboratory identified the human gene that causes cancer cells to resist many anticancer drugs by pumping these drugs out of drug-resistant human cancers and has used this information to create gene transfer vectors and to circumvent drug resistance in cancer.

In his job overseeing the intramural research program at the NIH, Michael has initated several training and mentoring programs for high school, college, post-baccalaureate, medical, and graduate students. He has also instituted training programs for students from disadvantaged populations, programs to advance the careers of women scientists, loan repayment programs for clinical researchers at NIH, and a clinical research training program for early career clinical investigators.

Alexandra Newton, Ph.D.,
Distinguished Professor of Pharmacology
University of California, San Diego

Dr. Newton received her PhD in Chemistry from Stanford University and, following two years as a postdoctoral fellow in the lab of Daniel E. Koshland, Jr., at the University of California, Berkeley, joined the Chemistry Department at Indiana University in 1988. She was recruited to the Department of Pharmacology at UCSD in 1995, where she is currently Distinguished Professor. Dr. Newton is recipient of a Du Pont Young Faculty Award, Searle Scholars Award, NSF Young Investigator Award, NSF Career Advancement Award, Outstanding Alumnus Award from Simon Fraser University, Avanti Award from the ASBMB, EC Slater Lectureship Award from the International Union of Biochemistry and Molecular Biology, Julius Axelrod Award in Pharmacology from ASPET and Biophysics of Health and Disease Award from the Biophysical Society. She is a fellow of the American Association for the Advancement of Science and received an NIH MERIT Award and currently has an NIH MIRA Award.

Dr. Newton has served as Chair of the Biomedical Sciences Graduate Program and is currently the Co-Director of the Molecular Pharmacology Training Area at UCSD. She is currently on the board of Scientific Directors of the National Cancer Institute. She was recently elected to lead the International Union of Biochemistry and Molecular Biology and is the first American woman to head this organization, which represents biochemical societies in 79 countries. She is Director a Cell Signaling San Diego; a center being created in 2020 that brings together the outstanding talent in San Diego in understanding cell signaling mechanisms. She is passionate about training the next generation of biochemists and biomedical researchers and has trained 28 PhD students and approximately that many postdoctoral fellows for successful careers in academia and industry.

Dr. Newton's research focuses on understanding of the structure, function, and regulation of a key signaling molecule in cells, protein kinase C, and how its function is altered in disease. Overturning a 30-year dogma, her recent analysis of mutations in protein kinase C isozymes found in various cancers revealed a tumor suppressive function of these enzymes, indicating that therapies should focus on restoring, rather than inhibiting, enzyme activity. Conversely, she showed that enhanced function of protein kinase C contributes to the pathophysiology of degenerative disease, identifying activity-enhancing variants of protein kinase C in patients with Alzheimer's Disease. Her work exemplifies how detailed dissection of the mechanisms of allosteric regulation of enzyme function provides the necessary biochemical understanding to drive effective therapeutic strategies.

Barbara Felber, Ph.D.
Senior Investigator of Vaccine Branch at NIH and Head of the Human Retrovirus Pathogenesis Section

Dr. Felber received her Ph.D. in molecular biology from the University of Bern, Switzerland. After carrying out postdoctoral studies in the Laboratory of Biochemistry, NCI Bethesda, she joined the Molecular Mechanisms of Carcinogenesis Laboratory, the NCI contract Basic Research Program, in 1985. In 1990, Dr. Felber established the Human Retrovirus Pathogenesis Group. In 1998, Dr. Felber received her tenure appointment and, in 1999, she joined the Center for Cancer Research, National Cancer Institute/NIH. Her work focuses on the posttranscriptional mechanisms of gene regulation, use of cytokines in cancer and AIDS, and the development of DNA-based HIV vaccines.

J. Carl Barrett, Ph.D.
Vice President at AstraZeneca, Oncology Translational Sciences,
Founding Director of CCR

Dr. J. Carl Barrett is Vice President & Global Head of Translational Medicine, Oncology R&D at AstraZeneca. He is responsible for development and execution of biomarker strategies and translational sciences efforts to support compound development from research through early and full development in oncology. From 2005-2011, he was Global Head of Oncology Biomarkers and Imaging at Novartis.

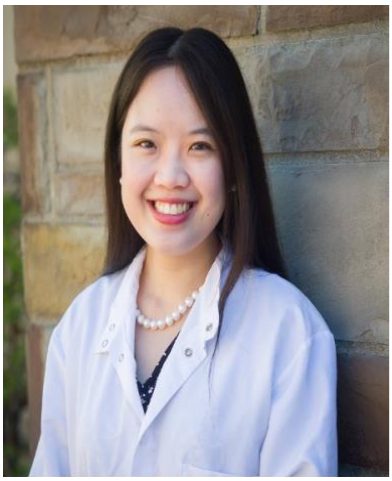
Dr. Barrett was the founding Director of the NCI Center for Cancer Research (CCR), the NCI intramural center for translation medicine. He was also Scientific Director at the National Institute of Environmental Health Sciences where he focused on integrating new approaches to toxicogenomics, molecular toxicology, and the Environmental Genome Project.

Dr. Barrett's research focused on the discovery of the critical genetic and epigenetic changes in the cancer cell, in particular the discovery of genes involved in breast cancer (BRCA1).

Trained as a chemist at the College of William and Mary, he received his Ph.D. degree in Biophysical Chemistry from Johns Hopkins University. He has published over 600 research articles. He is a member of the Johns Hopkins University Society of Scholars, the Ramazini Foundation, an honorary member of the Japanese Cancer Association, and a recipient of multiple NIH awards and Keynote lectures.

Outstanding Postdoctoral Fellow

Sachi Horibata, Ph.D.
Postdoctoral Research Fellow, Laboratory of Cell Biology
Center for Cancer Research, National Cancer Institute



Dr. Sachi Horibata received her Bachelor of Science degree in Biochemistry at the University of Wisconsin at Madison where she worked with Dr. Manish Patankar to understand the role of MUC16 in ovarian cancer. There, she developed a cell-cell interaction-based assay which she used to determine how immune cells target cancer cells. This led to four publications as an undergraduate researcher. She then pursued her Ph.D. at Cornell University under the guidance of Dr. Scott Coonrod, unraveling the role of peptidylarginine deiminase in chromatin decondensation and in mammary cancer progression. During this time, she also developed an interest in using genomic approaches to decipher the mechanism of tamoxifen resistance in breast cancer. She had 11 publications stemming from her graduate work and has received over 20 awards. Just to name a few, she was the winner of the 3-minute thesis competition at Cornell University, the recipient of the Bicknese prize award given to the most outstanding female scientist, and she was the first ever to receive a Daversa scholarship, which is given to one fellow each year who demonstrated the highest level of research productivity. She enjoys writing grant applications, which helped her receive numerous funding awards. Aside from her research accomplishments and devotion to cancer research, she deeply cares about cancer patients. She played a leading role in the creation of the Cornell Community Cancer Partnership, which connects Cornell cancer researchers with cancer patients and survivors within the community. The main goals of the Partnership are to provide support resources to cancer patients and survivors, and to enhance the overall training of cancer researchers. She was able to help secure funding to create a new four-course curriculum at Cornell university which provides cancer researchers with training in science communication and public engagement.

Dr. Horibata is currently a postdoctoral fellow in the laboratory of Dr. Michael Gottesman in the Laboratory of Cell Biology, National Cancer Institute, at the NIH. Her research focus is on understanding the mechanism of chemotherapy resistance in acute myeloid leukemia and in ovarian cancer. She is the recipient of the prestigious Japan Society for the Promotion of Science Postdoctoral Fellowship and the Sallie Rosen Kaplan Postdoctoral Fellowship. She won several awards and has been invited to give talks at international conferences. She recently served as a chair for the Cancer R&D conference.

Special Presentation: Cancer Survivorship

Marty Tenenbaum, Ph.D.
Computer Scientist and Cancer Survivor
Founder of Cancer Commons and CollabRx

Marty Tenenbaum is a renowned computer scientist, Internet entrepreneur and cancer survivor. He founded Cancer Commons, xCures and CollabRx (NASD: CRLX) to arm patients and their physicians with the knowledge they need to achieve the best possible outcomes and track their results to continuously learn. Dr. Tenenbaum began his career in AI, leading elite research groups at SRI International and Schlumberger Ltd. Later, as an Internet commerce pioneer, he founded or co-founded five successful startups. He is a fellow and former board member of the American Association for Artificial Intelligence, a former Director of Commerce One, the Public Library of Science (PLoS) and Patients Like Me, and a former consulting professor of Computer Science at Stanford. He currently serves as a Director of CommerceNet and Efficient Finance. Dr. Tenenbaum holds B.S. and M.S. degrees in Electrical Engineering from MIT, and a Ph.D. from Stanford, and has received numerous awards for his contributions to AI and his work as a patient advocate.

Workshops and Panel Discussions

Workshop- Management Techniques

Shannon Bell, MSW
Director of the NCI Office of Workforce Planning and Development
National Cancer Institute

Shannon K. Bell is the Director of the NCI Office of Workforce Planning and Development, where she leads the Workforce Development, Planning, and Engagement efforts across the NCI. Shannon has over 20 years' experience as a leader, manager, coach, facilitator, trainer, and mentor in the public sector. Shannon has a master's degree in Social Work and is certified as an executive coach and organizational development practitioner. Shannon fuels her passion by supporting individuals to enhance their positive impact on business through building collaborative relationships and teams, effectively managing difficult conversations, harnessing their strengths, and understanding and minimizing the impact of their weaknesses. Shannon is committed to leading an exemplary team of professionals who Advance People in order to Advance the Science.

Workshop- Virtual Interview Techniques

Scott Morgan, M.A.
Senior Associate, Center for Strategic and International Studies

Scott Morgan has been teaching leadership and communication skills for over 25 years. His clients include the majority of institutes at the NIH, Mount Sinai, the Mayo Clinic, the Howard Hughes Medical Institute and dozens of universities. Scott also teaches media and communication strategy to many think tanks and research organizations around the world. He is a Senior Associate at the Leadership Academy at the Center for Strategic and International Studies (CSIS), graduated with honors from the University of California Davis, and holds a master's degree in psychology from Columbia University. He authored the book *Speaking about Science* published by Cambridge University Press (2006) and launched a mindfulness app for young adults called *3rdi* in 2014.

Workshop- Networking and Scientific Communication

Phil Ryan, Ph.D.
Deputy Director of Graduate Programs and Student Services,
NIH Office of Intramural Training & Education

Dr. Phil Ryan currently serves as Deputy Director, Graduate Programs and Student Services. In this capacity he helps oversee the administration the NIH Graduate Partnerships Program as well as directing two graduate student summer programs: The Graduate Summer Opportunity to Advance Research (GSOAR) program and the Graduate Data Science Summer Program (GDSSP). Dr. Ryan also directs additional training programs for NIH postdocs, graduate students and postbacs, such as the Translational Science Training Program and the Intramural AIDS Research Fellowship. He also holds workshops and seminars on career and professional development topics. In his spare time, he teaches an online course on Cancer Biology through the University of Maryland, Office of Extended Studies. In every transition in his career, mentors played a key role in exploring options, discussing best choices and preparing him to make a successful transition. Dr. Ryan is passionate about helping others find mentors and make the most out of the mentoring relationships.

Academia Panel

Alexandra Newton, Ph.D.,
Distinguished Professor of Pharmacology
University of California, San Diego
For more information, see the "Keynote Speakers" section

Kirill Afonin, Ph.D.
Associate Professor, Department of Chemistry
UNC-Charlotte

Dr. Afonin graduated from Saint Petersburg State University with a M.S. in Chemistry, followed by a Ph.D. in Photochemistry earned from Bowling Green State University, Ohio. In addition, he also obtained a Graduate Certificate in Bioinformatics, Proteomics/Genomics. In the following three years, Dr. Afonin completed a Postdoctoral Fellowship in Chemistry and Biochemistry at the University of California Santa Barbara. In 2011, he was invited as a Research Fellow to the National Cancer Institute, NIH where he established and managed an experimental branch within the Computational RNA Structure Group. He started his tenure-track appointment at UNC Charlotte in 2015, was promoted with permanent tenure to the rank of Associate Professor in 2019, and to the rank of full professor in 2021. Dr. Afonin currently serves as a founding council member and vice-president of International Society of RNA Nanotechnology and Nanomedicine. Among other awards, he is a recipient of two NIH Fellows Awards for Research Excellence (FARE), a prestigious NIH Maximizing Investigators' Research Award (MIRA R35), and two NIH R01s.

Emma Benn, DrPH,
Associate Professor, Center for Biostatistics and Department of Population Health Science and Policy
Icahn School of Medicine at Mount Sinai

Dr. Benn is an Associate Professor in the Center for Biostatistics and Department of Population Health Science and Policy at the Icahn School of Medicine at Mount Sinai (ISMMS). She is also the Founding Director of the Center for Scientific Diversity and Associate Dean of Faculty Well-being and Development at ISMMS. She is also the Director of Data Science Training and Enrichment in the Graduate School of Biomedical Sciences and serves as a member of the Faculty Diversity Council, Quality Leadership Council, and Anti-racism Task Force at ISMMS. Dr. Benn is the former co-Director of the Master of Science in Biostatistics Program and former Director of Academic Programs for the Center for Biostatistics at ISMMS. Dr. Benn has collaborated on a variety of interdisciplinary research projects over the course of her career and is particularly interested in health disparities research. She teaches a graduate-level course, Race and Causal Inference, designed to increase the methodologic rigor by which students and trainees investigate health disparities with a goal of finding effective causal targets for intervention. Dr. Benn, through her leadership of the Center for Scientific Diversity, is committed to increasing diversity, inclusion, and equitable advancement in (bio)statistics and the biomedical research workforce, more broadly, as well as reducing racial/ethnic disparities in faculty promotion in academic medicine. Dr. Benn is the co-founder of the NHLBI-funded Biostatistics Epidemiology Summer Training (BEST) Diversity Program and a former co-Chair of the ENAR Fostering Diversity in Biostatistics Workshop. She currently serves on the American Statistical Association's Task Force on Antiracism and LGBTQ Advocacy Committee. She also serves as a mentor for the JSM Diversity Workshop and Mentoring Program and the Math Alliance. Dr. Benn was co-PI of the NIGMS-funded Applied Statistics in Biological Systems (ASIBS) Short Course aimed at increasing the statistical competency and research capacity of early-stage researchers nationwide. She currently is co-PI of the NHGRI-funded Clinical Research Education in Genome Science (CREiGS) Short Course aimed at exposing doctoral students, postdocs, and clinical and research faculty to computational tools in genome science in addition to effective strategies for engaging underserved communities in genomics research. Dr. Benn's contributions to diversity and inclusion in statistics and STEM have been celebrated by various organizations including Mathematically Gifted and Black, Graduate Women in Science, and the American Statistical Association.

Regina Nuzzo, Ph.D.
Professor, Department of Science, Technology & Mathematics
Gallaudet University

Dr. Regina Nuzzo is a freelance science writer and professor in Washington, DC. After studying engineering as an undergraduate she earned her PhD in Statistics from Stanford University. Currently she's teaching statistics in American Sign Language at Gallaudet University, the world's only liberal arts college for deaf and hard-of-hearing students.
Dr. Nuzzo is also a graduate of Science Communication program at the University of California-Santa Cruz. Her science journalism specialties center around data, probability, statistics, and the research process. Her work has appeared in Nature, Los Angeles Times, New York Times, Reader's Digest, New Scientist, and Scientific American, among others. You can read some of her writing here.

Dr. Nuzzo has been invited to speak to a variety of audiences about her work, such as why we just can't understand p-values, how our brain can fool us during data analysis, what happens when people abuse and misuse statistics, and tips and tricks for communicating anything with numbers and statistics. You can read more about some of her talks here.

Non-Profit Organization Panel

Laurel Oldach, Ph.D.,
Scientific Communicator at the American Society for Biochemistry and Molecular Biology

Laurel Oldach is a science writer at the American Society for Biochemistry and Molecular Biology. She covers research news, science careers and the biotechnology/pharmaceutical industry for the society's member magazine, ASBMB Today, and previously worked in communications for the society's journals. Prior to being hired at ASBMB, she studied biology at Reed College and earned a Ph.D. in biochemistry, cellular and molecular biology from Johns Hopkins University.

Richard Turman, M.P.P.,
President at ACT for NIH

Before joining ACT for NIH in January 2020, Richard Turman held multiple leadership positions on NIH budgeting and advocacy. He served as NIH's Associate Director for Budget, helping NIH make the case for continued investments in life-saving research. He advocated for the completion of NIH's doubling while leading the federal relations activities of the Association of American Universities. And he helped develop a decade's worth of NIH budgets while working as a program examiner and branch chief at the White House Office of Management and Budget.

Turman has also held senior executive positions in the Federal and non-profit sectors, serving as the HHS Deputy Assistant Secretary for Budget, the HHS Principal Deputy Assistant Secretary for Financial Resources, the Deputy Director of FDA's Center for Tobacco Products, and most recently as the Chief Operating Officer of the People-Centered Research Foundation.

Turman has worked in the California legislature and in the United States Senate, both times for appropriators. He earned a B.A. in economics and history from the University of California, Santa Cruz, and a Master of Public Policy from the University of California, Berkeley.

Lynn Marquis, B.A.
Director at the Coalition for the Life Sciences

Lynn Marquis represents the interest of the Coalition for the Life Sciences (CLS) and its over 60,000 members before Members of Congress and leaders in the Administration. She is primarily responsible for educating political leaders on the need for a robust federally funded scientific enterprise. Lynn serves in a leadership capacity on many boards and committees that share the unified commitment for advancing the U.S. biomedical research enterprise.

Lynn has over 20 years of experience in Washington, DC. Beginning her career on Capitol Hill as a legislative aide to then Representative Bill Brewster (D-OK), she went on to work as a lobbyist on behalf of many health care interest before fine tuning her experience on behalf of the nation's scientific enterprise.

Lynn has a B.A. in Communications and Political Science from the University of Southern Maine.

Erin Rosenbaugh, Ph.D., P.M.P.
Associate Scientific Project Manager for Neuroscience
Foundation for the National Institutes of Health

Dr. Erin Rosenbaugh is an Associate Scientific Project Manager for Neuroscience at the FNIH. She supports established and developing and projects focusing on neurodegenerative diseases and neuropsychiatric disorders within the Biomarkers Consortium. Before joining FNIH, Erin was most recently a Scientific Project Manager for Cape Fox Corporation where she recruited and managed multidisciplinary federal contractors for the National Institutes of Health (NIH) Scientific Technical and Other Professional Support Services (STOPS) Program as well as launched operations for a National Institute of Allergy and Infectious Disease NIAID Professional Scientific Technical Support Services (PSTSS) Contract. She performed clinical drug testing as a Confirmation Scientist for Burlington Labs (now Aspenti Health) and served as an Adjunct Faculty Instructor for Champlain College in Burlington, Vermont. Erin received her Ph.D.in Cellular and Integrative Physiology from the University of Nebraska Medical Center and her Bachelor of Science in Biotechnology from the University of Nebraska Omaha.

Drug Development and Industry Panel

J. Carl Barrett, Ph.D.
Vice President at AstraZeneca, Oncology Translational Sciences,
Founding Director of CCR
For more information, see the "Keynote Speakers" section

Scott Martin, Ph.D.,
Director, Functional Genomics & Principal Scientific Manager
Department of Discovery Oncology, Genentech

I lead numerous functional genomics efforts at Genentech where my group conducts chemical and genetic screens to interrogate basic biology and therapeutically relevant questions for the Genentech community. These efforts span all therapeutic areas and range from genome-wide screens to much more focused efforts in complex model systems. Prior to joining Genentech in 2015, I established a functional genomics facility for the U.S. National Institutes of Health. There, my group collaborated with numerous NIH institutes to understand gene function in diverse areas, including oncology, infectious disease, and neuroscience. I have also remained focused on understanding and improving best practices for functional genomics, and related model and assay technologies.

Nicole Schiavone, Ph.D.
Senior Scientist
Pfizer

Dr. Schiavone earned her B.S. in Chemistry and Biochemistry from La Salle University in 2012. She attended the University of Notre Dame for graduate school, where she developed a capillary electrophoresis-mass spectroscopy (CE-MS) workflow for metabolomics using an in-house CE-MS interface. In 2017, she was awarded her Ph.D. in Analytical Chemistry. Upon graduation, she completed a postdoctoral fellowship at Merck focused on applying HDX-MS and circular dichroism to study global conformational changes of peptides in solution. In 2018, she joined Pfizer as a Senior Scientist in the Biotherapeutics Analytical Research & Development department where she plans and executes chromatography-based method development and provides project support for several molecular modalities. Outside of work, she is a 200-hour registered yoga teacher and enjoys staying active outdoors!

Matthew Meyer, Ph.D.
Senior Director and Head
Discovery Pharmacology and in vivo Biology
Bristol Myers Squibb

Dr. Matthew Meyer attended Kansas State University for his undergraduate and early graduate studies, earning his B.S. (1998) and M.S. (2000). He continued his graduate education at Cornell University where he was awarded his Ph.D. in 2005. Upon graduation, he completed a postdoctoral fellowship at the NCI in the Barbara Vonderhaar laboratory. In 2010, he joined Novartis as an Investigator I in the Oncology Pharmacology Department. Over the course of nine years at Novartis, Matt took on increasing responsibilities including leadership of drug discovery programs and becoming a member of the Oncology Pharmacology Leadership Team. During this time, he led or contributed to biologic and small molecule drug discovery programs from hit ID through IND filing & first in human trials. In 2019, he moved to Bristol Myers Squibb where he is a Senior Director, Head of the Discovery Pharmacology and in vivo Biology Group, and a member of the Cambridge Oncology Thematic Research Center Leadership Team.

Abstracts for Oral Presentations

Bioinformatics, Epidemiology and Translational Research

Genomic and transcriptomic profiling of malignant mesothelioma patients identifies gene signatures predictive of survival and response to immuno and chemotherapy

Nishanth Ulhas Nair[1#], Qun Jiang[2#], Jun Stephen Wei[3#], Vikram Alexander Misra[2], Betsy Morrow[2], Leandro C. Hermida[1,4], Joo Sang Lee[5], Idrees Mian[2], Jingli Zhang[2], Manjistha Sengupta[2], Javed Khan[3], Eytan Ruppin[1]*, Raffit Hassan[2]

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Objectives
Malignant mesothelioma (MM) is an aggressive cancer with limited treatment options and poor prognosis. Malignant pleural mesothelioma comprises 80% of the cases and has worse outcome than malignant peritoneal mesothelioma. An in-depth knowledge of genetic, transcriptomic and immunogenic events involved in MM is critical for successful development of prognostics and therapeutic modalities. In this study we aim to address this by exploring a new large scale patient tumor dataset containing both pleural and peritoneal mesothelioma patient samples.
Methods
We performed whole-exome sequencing of germline and tumors of 122 patients with pleural (n=59), peritoneal (n=61) and tunica vaginalis (n=2) mesothelioma, and RNA-sequencing of 100 tumors to identify pathogenic variants, somatic mutational signatures, and prognostic gene expression signatures, predictive of patient survival and tumor response to therapies. We validated our findings using the TCGA and Bueno et al. mesothelioma datasets.
Results
The important findings from this study include:
a) Key somatic mutational signatures are associated with DNA repair pathways and BRCA1 associated protein-1 (BAP1) is the most commonly mutated gene (~13% with germline mutation). b) Unlike previous studies which mainly focused on pleural mesothelioma patients, our dataset includes comparable number of patients of both pleural and peritoneal subtypes, and hence give us a better understanding of the similarities and differences that may exist in the molecular pathophysiology of the two anatomically distinct disease. c)We identified a set of 48 genes, a ‚Äúmesothelioma prognostic signature‚Äù, whose high expression level is associated with poor survival (Cox regression, FDR < 0.1). These genes are enriched for genes related to cell cycle and DNA repair. This signature is highly predictive of patient survival in two other independent, pleural mesothelioma cohorts: TCGA (Hazard ratio (HR) = 2.6, P = 6.94e-10) and Bueno et al. mesothelioma dataset (HR = 1.49, P = 4.34e-07), after controlling for age and gender. d)Among the 48 genes, the expression of CCNB1 is highly predictive of patient survival suggesting its important role in MM, possibly via its involvement in the CDK1-CCNB1-CCNF complex (HR = 2.54, P = 1.89e-08 for TCGA; HR = 1.40, P = 1.65e-05 for Bueno et al. dataset). e) Using a synthetic lethality (SL) based precision-oncology computational framework for analyzing the patients, ‚Äô transcriptomic data, we were able to accurately predict response to an anti-PD1 immune checkpoint inhibitor and combination therapies with pemetrexed (chemotherapy) in mesothelioma patients. The SL profiles successfully predicted the overall patient-response observed across targeted, immuno- and chemotherapies in 11 independent mesothelioma clinical trials (Spearman‚Äôs œ = 0.64, P = 0.0348). This is the first analysis shown to successfully predict overall patient-response for various treatments within a cancer type.
Conclusions
By analyzing the tumor genomic and transcriptomics data of a large cohort of MM patients, we identify gene expression prognostic markers predictive of patient survival and response to therapy, both as independent signatures and via their SL interactions. These findings lay a basis for the future development of personalized therapy approaches for mesothelioma patients.

de novo identification of cell-type specific intracellular microbes from single-cell RNA-seq data

Welles Robinson[1,2], Fiorella Schischlik[1], E. Michael Gertz[1], Max Leiserson[2], Alejandro A. Schaffer[1], Eytan Ruppin[1]

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A long-standing challenge in the study of the microbiome is distinguishing between extracellular, intracellular and contaminating microbes. We hypothesize that intracellular microbes that preferentially reside in a given cell-type (cell-type specific intracellular microbes) can be identified de novo using both plate and droplet-based single cell RNA-sequencing (scRNA-seq). We first analyze datasets where scRNA-seq is performed on immune cells exposed to the intracellular bacteria Salmonella and find that infected cell-types can be distinguished from bystander cell-types by differential presence (the proportion of cells with reads mapped to Salmonella) for droplet-based protocols and differential abundance (the number of reads mapping to Salmonella per cell) for plate-based protocols. We leverage these observations to build a computational pipeline CSI-Microbes (computational identification of Cell type Specific Intracellular Microbes) for the de novo identification of cell-type specific intracellular microbial taxa from human single-cell RNA-sequencing. On the gold-standard datasets, CSI-Microbes identifies Salmonella but none of the other microbial taxa identified from unmapped reads to be a cell-type specific intracellular microbe, demonstrating the ability to distinguish true intracellular bacteria from contaminants. We further show that microbial reads can be used to identify host pathways including antigen processing and presentation that are downregulated by infection and identify microbial genes associated with the perturbation of specific human genes. Next, we apply CSI-Microbes to droplet-based scRNA-seq datasets from Merkel cell and colorectal carcinoma and identify previously reported tumor-cell enrichments of Merkel polyomavirus and Fusobacterium respectively as well as the novel enrichment of the bacterial species Hathewaya histolytica in tumors cells from one colorectal carcinoma. Next, we apply CSI-Microbes to a recently published plated-based scRNA-seq dataset from lung cancer patients and identify four tumors where specific bacterial taxa are enriched in the tumor cells and two tumors with specific bacterial taxa enriched in other cell-types. In particular, we identify the lung commensal and known intracellular bacteria species Cutibacterium acnes to be enriched in the tumor cells of these four lung tumors. Finally, we compare the transcriptome of the tumor cells with and without putative intracellular bacteria and find infected cells strongly downregulate pathways associated with response to cytokines, defense against bacteria invasion and antigen processing and presentation. These results demonstrate the ability of CSI-Microbes to identify candidate intracellular bacteria living within specific cell populations in tumors, complementing previous studies inferring microbial abundance from bulk tumor expression data on a more refined, single cell resolution.

Immune cell transcriptomic signatures in different cancer types markedly diverge from their corresponding signatures in the peripheral blood

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Recent advances towards elucidating the role of immune cells in the tumor microenvironment have led to the development of numerous computational tools to quantify the abundance of immune cells within a given tumor. Such tools, e.g., the widely used CIBERSORT, frequently use transcriptomic signatures (e.g., LM22) derived from sorted cell populations of peripheral blood, assuming that they are representative of the transcriptional state of resident tumor immune cells.

Here we systematically derive de novo immune cell transcriptomic signatures from peripheral blood and tumor tissues and rigorously quantify their similarity. To this end we leveraged upon the recently published TISCH dataset, which re-processed single cell datasets from several cancer types as well as from peripheral blood of healthy donors. We derived the signatures of 6 key immune cells (Monocytes/Macrophage, NK, DC, CD4Tconv, CD8T and B cells) across 7 different cancer types. In short, for each patient/sample (n=45), the signatures were derived by extracting the genes that are significantly overexpressed in one cell types versus the other cell types. We choose the optimal number of signature genes which form an expression matrix with the lowest condition number calculated by a kappa function.

Having inferred the immune cell signatures for each patient, we estimated the similarity between these signatures in the blood and those of each cancer type, by computing the mean similarity between all sample in the blood and those of a given tumor type. Similarity was measured as the Jaccard overlap between the sets of genes composing the blood and cancer type immune cell signatures, resulting in a total of 48 (7 cancers + 1 blood x 6 cell types) similarity coefficients. We find a large variation in the similarity levels of resident immune cells from their blood counterparts, for different cell types: B cells and NKs showed the highest similarity to blood and while CD4T and C8T cells were the most divergent in their transcriptomics. Examining the immune transcriptomic divergence across cancer types shows less variability, however, some cancer types such as liver cancer and Merkel cell carcinoma immune signatures are more similar to those in the blood, while skin cancers (SKCM and BCC) were the most divergent. Notably, CD8T cells signatures were the most divergence across almost all tissues.

In summary, our results show that the transcriptomic signatures of resident immune cells in tumors vary considerably from those observed in the peripheral blood and provide their first systematic characterization. These findings, if further validated, have important biological ramifications. From a computational perspective, they put forward the need for incorporating tumor type specific immune signatures in future deconvolution studies.

TNIK, a novel activator of FAK and YAP signaling, is a therapeutic target in Lung Squamous Cell Carcinoma

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Distal chromosome 3q amplification (3q26-29, also known as the 3q amplicon) is the most frequent genomic alteration in lung squamous cell carcinoma (LSCC). Analysis of LSCC samples from the TCGA reveals that nearly 50% of LSCC patients harbor the 3q amplicon that includes the resident protein kinase gene TNIK. Recent studies have pinpointed TNIK as a potential oncogenic driver in cancer cells with distal 3q amplification; however, the therapeutic potential of TNIK remains unexplored.
We found that TNIK was highly expressed in LSCC cells with the 3q amplicon, while its expression was modest in cells that lacked the 3q amplicon, consistent with data from the TCGA. To evaluate TNIK as a target in LSCC, we generated stable, doxycycline-inducible cells expressing shRNA to deplete TNIK from LSCC cells and conducted functional assays to measure cell proliferation and survival. TNIK knockdown or inhibition of its kinase activity with a small molecule inhibitor significantly diminished the viability of LSCC cells with 3q amplification in vitro and in cell line-derived xenograft mouse models. We also observed that TNIK inhibitors significantly abrogated the growth of LSCC patient-derived xenografts and showed that TNIK inhibition induced apoptotic cell death in LSCC cells that harbor the 3q amplicon. Importantly, TNIK depletion or catalytic inhibition in LSCC cells that lack the 3q amplicon had no significant effect on cell survival.
Finally, we used a combination of bioinformatics and proteomic analysis (RPPA, peptide mapping, and mass spectrometry) to define the underlying mechanisms driving TNIK mediated cancer cell survival. We identified the tumor suppressor MERLIN as a novel TNIK substrate and determined that TNIK phosphorylates MERLIN at serine 13 and 315. We also show that TNIK is required to maintain FAK activation and stabilize the YAP transcription factor, two oncogenic pathways inhibited by MERLIN.
In conclusion, our results demonstrate that TNIK maintains survival of LSCC cells through modulation of a novel TNIK-MERLIN-YAP/FAK signaling pathway and validate TNIK inhibitors in pre-clinical models of LSCC, including patient-derived xenografts. In summary, we have pinpointed the protein kinase TNIK as a promising therapeutic target for the treatment of LSCC patients with distal chromosome 3q amplification.

Biophysics, Chemistry, Pharmacology and Structural Biology

Assessing the NRAS 5' UTR as a target for small molecules to control the NRAS expression

Sumirtha Balaratnam[1*], Zachary Torrey[2], David R. Calabrese[3], and John S. Schneekloth Jr[1]
Chemical Biology Laboratory, National Cancer Institute, Frederick, MD 21702, USA[1]

Neuroblastoma RAS (NRAS) is an oncogene that is deregulated and highly mutated in several cancers, notably 15% to 20% in Melanomas, Acute Myeloid Leukemias and less commonly in colon adenocarcinoma, thyroid carcinoma, and other hematologic malignancies. Constitutively activated NRAS mutations induce mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase,ÄìAKT pathway signaling and drive malignant progression. The activated NRAS oncogene in human malignancy has proved to be an elusive target. However, like all RAS-family proteins, attempts to directly target NRAS with small-molecule inhibitors have been largely unsuccessful due to its small size and a single binding pocket with a picomolar affinity for GTP. To date, the promising strategies to target NRAS include targeting the membrane localization of NRAS or using the inhibitors which target downstream signaling through mitogen-activated protein kinase kinase and phosphatidylinositol 3-OH kinase or AKT. An alternative approach would involve targeting the NRAS mRNA. Our current efforts have focused on targeting a structure found in its mRNA and preventing the NRAS protein from even being produced. The 5,Äô untranslated region (5,ÄôUTR) of the NRAS mRNA is reported to contain a non-canonical secondary structure G-quadruplex (GQ), that regulates the translation process of NRAS mRNA. Stabilizing the GQ structure in NRAS by small molecules provides an alternative approach to reduce NRAS expression in cancer cells. However, a major barrier in developing biologically active small molecules that bind to nucleic acids has been the identification of selective interactions. Previous approaches have generally yielded pan-GQ binding molecules, and strategies to generate selective ligands are lacking. Here we use a small molecule microarray screen to identify a small molecule that selectively bind to the GQ located 5,ÄôUTR of the NRAS mRNA. Biophysical studies, including thermal melt, fluorescence titration and SPR analysis, demonstrate that the compound binds reversibly to the NRAS GQ structure with nanomolar affinity with weaker or no measurable binding to several other GQs. A Luciferase based reporter assay indicated that one compound inhibits the translation of NRAS via stabilizing the NRAS-GQ. Structure probing and sequencing analysis provide further insights into the structure and targetability of the 5,Äô UTR. We demonstrate that the SMM approach can reveal a selective GQ binder for oncogene inhibition. Efforts toward applying SMMs to other GQ-associated oncogenes are being pursued to discover new selective binding scaffolds.

Development and characterization of PIM1 degraders as a new targeting strategy

Sophia A. Lookingbill[1], Pedro Torres-Ayuso[1], Ryan C. Bensen[1], Venkata Sabbasini[2], Rolf Swenson2, John Brognard[1]

[1] Laboratory of Cell Developmental Signaling, National Cancer Institute

PROTACs are bifunctional compounds containing a pharmacophore directed against a target of interest and an E3 ubiquitin ligase warhead connected by a linker. PROTACs work via the ubiquitin-proteosome system. The pharmacophore identifies the target protein, which is then ubiquitinated and tagged for proteasomal degradation, and the PROTAC is recycled for a new cycle of targeted degradation. As a result, one PROTAC molecule can degrade several target molecules and be more efficacious than catalytic inhibitors, which work in a 1:1 target-to-inhibitor ratio. Moreover, because PROTACs function by reducing the target‚Äôs levels, PROTACs downmodulate both enzymatic and non-enzymatic functions of proteins, providing an additional advantage over small molecule inhibitors.

The Proviral Integration for the Moloney murine leukemia virus (PIM) kinases are a family of serine/threonine kinases. [The PIM kinases are unique to other kinases as they lack regulatory domains and stay active after translation. Therefore, the functionality of PIM kinases primarily relies on the variation of ubiquitination and proteasomal degradation rates.] PIM kinases function in cell cycle and metabolic regulation, as well as angiogenesis and drug resistance. Of the three isoforms in the PIM kinase family, PIM1 is a prognostic indicator in multiple solid-tumor cancer types, including prostate, breast, and pancreatic cancers. As a result, PIM inhibitors are in clinical trials. However, PIM kinases also show non-catalytic functions, making PIM1 a target for Proteolysis Targeting Chimeras (PROTACs) development.

Four PIM1 PROTACs were synthesized by coupling a PIM1 inhibitor to different E3-ubiquitin ligase ligands (Von-Hippel Lindau [VHL], Cereblon [CRBN], and Inhibitors of Apoptosis Proteins [IAP]) that differ in their linker. The resulting PIM1 PROTACs were investigated for timed maximal PIM1 degradation, validation of the mechanism of action, and comparison of induced and endogenous conditions.

We found a preference for the VHL PROTACs over the IAP PROTAC, which triggered PIM1 degradation in the low micromolar range. No degradation was observed with the CRBN PROTAC. In time course experiments we observed efficient PIM1 degradation up to 72 hours after treatment. The PROTAC mechanism of action was confirmed by the administration of proteosome and neddylation inhibitors which rescued PIM1 expression. Ongoing experiments will further verify the specificity of the VHL-coupled PIM1 PROTACs over PIM2 and PIM3 and evaluate the efficacy of PIM1 PROTACs versus inhibitors to reduce cell viability.

We have identified a lead PROTAC that triggers PIM1 kinase degradation in the low micromolar range. Future projects will aim to improve the efficacy of this compound. Because PIM1 promotes tumorigenesis and drug resistance through catalytic and non-catalytic mechanisms, targeting both functions with novel compounds, like PROTACs, is a promising step toward improved clinical applications.

Mapping mechanical changes during extravasation into brain in vivo danio rerio (zebrafish)

Woong Young So, Zhilin Yang, Patricia B. Gordon, Colin D. Paul, Perry Lim, and Kandice Tanner
Tissue Morphodynamics Unit, Laboratory of Cell Biology, National Cancer Institute, National Institute of Health, Bethesda, Maryland, USA

Patients suffering from brain metastasis have especially poor prognosis with high morbidity and mortality compared to patients showing other sites of metastasis. Despite recent improvements in systemic disease treatment and associated brain metastases, the median patient survival of metastatic brain lesions is approximately 7-16 months from diagnosis. Poor prognosis is underscored by the fact that these patients have also a reduced quality of life. Understanding factors crucial to cancer cell survival following dissemination is critical for developing optimal treatments for metastatic and resistant tumors.

In patients, brain metastases mostly derive from breast cancer, lung adenocarcinoma, and melanoma. Here, we focus on breast cancer by using a human breast cancer cell model of metastasis in danio rerio (zebrafish). We wish to understand how cells can leave the blood vessels and extravasate into the brain. We reason that cancer cells need to tune their mechanical properties to be able to squeeze through the endothelium while also resisting the shear stress of blood flow. We further propose that if cells cannot mechanically adapt, they will remain in the luminal spaces of the blood vessels.

In the last five to six years, zebrafish has rapidly become a model for studying tumor behavior at different stages of the metastatic cascade. Optical transparency needed for single cell imaging and homology between human and zebrafish proteins are often described as strengths for use of this model. Furthermore, recent studies have demonstrated that multiple brain-homing human breast cancer cell lines also home to the zebrafish brain suggesting that this process is sufficiently conserved in zebrafish. Based on in vivo danio rerio (zebrafish) brain model, the extravasation process also can be quantitated by optical tweezer based active microrheology. Human xenografts in zebrafish enable quantification of the mechanical properties of cells in terms of pre-extravasation and post-extravasation after injection in the circulation of zebrafish.

During brain metastasis, cancer cells encounter the first mechanical barrier of blood brain barrier before extravasation. In vitro models have shown that tumor cells go through huge deformation during extravasation. Here we provide in vivo data showing that the cancer cells become softer after extravasation in comparison to the pre-extravasation. The softening phenomena was also observed from our extravasation-mimicking microfluidics device in the function of ‚Äòin-channel‚Äô and ‚Äòpost-channel‚Äô. We investigated the role of yes-associated protein (YAP), a mechano-transducer protein, during extravasation with the device. Cancer cells pre-treated with YAP inhibitor could not extravasate, demonstrating that mechano-regulatory mechanism is important during metastasis. Finally, we followed individual extravasated cancer cells to observe that they stiffened after one day-post-extravasation from in vivo data. Future studies will examine the cytoskeleton to determine what molecular determinants drive this cellular mechanical change or adaptation in vivo.

RNA structural determinants of the binding interactions between HNRNPH1 and its target sites at the 3,Äô end of EWSR1-exon 8 present in the oncogenic fusion pre-mRNA expressed in a subset of Ewing sarcomas

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RNA maturation is a multistep process that involves the interaction of the many RNA-binding proteins that regulate mRNA biogenesis, including those involved in exon inclusion or exclusion, with specific RNA sequences or complex secondary or tertiary structures. About two-third of Ewing sarcomas (EWS) harbor a translocation that fuses a portion of the EWSR1 gene to the 3' end of the FLI1 gene. This fusion oncogene, referred to as EWS-FLI1, expresses the fusion oncoprotein EWS-FLI1 that promotes EWS tumorigenesis. We recently identified the RNA-binding protein HNRNPH1 as essential for excluding EWSR1-exon 8 from the nascent RNA expressed in approximately one-third of EWS-FLI1 driven tumors. Failure to exclude EWSR1 exon 8 (181 nucleotides, nts) results in the expression of an mRNA that includes a premature stop codon and a loss of the expression of the EWS-FLI1 oncoprotein EWS cells depend on for survival. We previously showed that HNRNPH1 binds to at least one G-rich sequence present in the 3' end of EWSR1-exon 8. In the presence of monovalent cations, G-rich nucleic acid sequences (RNA and DNA) can form noncanonical secondary structures called G-quadruplexes (G4s). We are investigating if: 1) HNRNPH1 can bind to multiple G-rich sequences at the 3' end of EWSR1-exon 8, and 2) whether the G-rich sequences' structural conformations influence the biophysical properties of the interactions with HNRNPH1.

In this study, we designed G-rich RNA oligomers corresponding to sequences present at the 3' end of EWSR1-exon 8 ,Äì rG1 (25 nts) and rG3b (29 nts) ,Äì and the variants of these sequences, rG1-s and rG3b-s, respectively. These variants contain a chemically modified version of guanosine (8-aza-7-deazaguanosine, or super G, IDT) that retain the same sequence as the wild-type oligomers, but eliminate the Hoogsteen hydrogen bonds required for the formation of a G4 structure in the presence of a monovalent cation. Circular Dichroism (CD) study shows rG1 and rG3b can form stable parallel quadruplex structures under physiological relevant salt conditions, while rG1-s and rG3b-s resemble the unfolded structure under similar conditions. Electrophoretic mobility gel shift assay (EMSA) shows HNRNPH1 interacts with both G4 and unfolded oligomers, but with different shift profiles. Biolayer Interferometry (BLI) results show HNRNPH1 binds to rG1 and rG3b with similar dissociation binding equilibrium constants (KD ~ 4-10 nM, 150 mM KCl) and sub-nanomolar KD values for the interactions with rG1-s and rG3b-s. Preliminary kinetic parameters obtained from the protein-RNA interactions at 150 mM KCl show differences between association (on-rate) and dissociation (off-rate) when HNRNPH1 interacts with wild-type vs. mutant sequences. Thermodynamic and kinetic parameters observed from protein-RNA interactions indicate RNA structural conformations (folded G4 and unfolded) may influence the dynamic interactions between HNRNPH1 and its target RNA sequences. These and future biophysical studies will help elucidate determinants of the alternative splicing of EWSR1-exon 8 and enhance our understanding of the biological importance of RNA G-quadruplex structures in regulating RNA maturation.

Cancer Models, Cancer Stem Cells, Carcinogenesis and Metastasis

Generation of PD-L1 nanobody-based CAR T cells for solid tumors

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Background and Hypotheses: Chimeric antigen receptor (CAR)-T therapy has shown great potency against hematological malignancies, while it is difficult to translate CAR T into solid tumors due to lack of tumor-specific targets and immunosuppressive tumor microenvironment (TEM). Checkpoint molecule PD-L1 was widely overexpressed on multiple tumor types, and PD-1/PD-L1 interaction is a primary mediator of immunosuppression in the TEM, which may be one of the mechanisms for the poor effect of CAR T in solid tumors. Clinically, antibody-based PD-1/PD-L1 antagonists were reported to induce durable tumor remissions whereas sometimes remains poor in advanced solid tumor. Here, we hypothesize that CAR T cell strategy targeting PD-L1 might be an effective method to modulate the TEM and treat solid tumors. We developed PD-L1-specific shark nanobody (VNAR)-based CAR T cell and explored its anti-tumor efficacy in xenograft mouse models of triple-negative breast cancer (TNBC) and liver cancer.
Study design and Methods: Phage display was performed to isolate anti-PD-L1 nanobodies from an engineered shark VNAR single domain library. Specific binding of nanobodies was validated by ELISA and flow cytometry. Cytolytic activity of PD-L1-targeted CAR T cells in vitro was determined via cell luciferase killing assay. In vivo activity was evaluated in MDA-MB-231 and Hep3B xenograft mouse models. Additionally, bi-specific CAR T cells targeting both GPC3 and PD-L1 were produced by co-transducing CAR vector lentivirus of an anti-GPC3 specific hYP7 single chain Fv and an anti-PD-L1 B2 shark VNAR.
Results and Conclusions: We successfully constructed an engineered shark single domain phage library with the size of 1.2x1010 PFU/ml. Three high-affinity anti-PD-L1 phage binders, B2, A11, and F5, were isolated from this phage library. They showed cross-activity to both human and mouse PD-L1 antigen, whereas B2 functionally blocked the interaction of human PD-1 to PD-L1. Moreover, we showed CAR (B2) T cells specifically lysed PD-L1-positive tumor cells in vitro, lead to tumor regression and eliminated lung/liver metastases in an MDA-MB-231 orthotopic xenograft mouse model. Importantly, CAR (B2) T cells showed a potent antitumor effect towards Hep3B tumor in vitro and in vivo through targeting inducible expression PD-L1 on tumor cells. In addition, bi-specific CAR (hYP7-B2) T cells targeting both GPC3 and PD-L1 triggered robust cytotoxicity against Hep3B cells. We concluded that the PD-L1-targeted shark nanobody-based CAR T cell is a promising strategy to treat constitutive PD-L1 expressed triple-negative breast cancer and liver cancer.
Relevance and Importance: This work provides a rationale for the potential use of anti-PD-L1 CAR T cells as a monotherapy or combination with a tumor-specific therapy in clinical studies for solid tumors.

Differential requirement of BRCA2-DSS1 interaction in mitotic and meiotic homologous recombination

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Introduction- BRCA2 is an important player in DNA repair via homologous recombination. It helps loading RAD51, a key DNA repair protein, on the resected ends of the damaged DNA removing RPA nucleofilaments with the help of DSS1 bound to its oligo binding domain. This BRCA2-DSS1 interaction is considered to be indispensable for RAD51 loading and eventual homologous recombination. Mutations in the DSS1 binding domain of BRCA2 are known to be pathogenic as that weakens the BRCA2-DSS1 interaction, one such mutation is BRCA2L2510P. One family had two live births inheriting this variant along with a truncating mutation p.E1550X, although they died within few years because of Fanconi anemia. How does such a deleterious mutation support survival intrigued us to study this mutation.

Results- We generated a mouse model of this mutation and observed that hemizygous or homozygous mice have no birth defects and survive normally. However, the pups born to the heterozygous parents has a skewed genotypic ratio, i.e. very few homozygous pups are born. The mutants which are born are viable and fertile and are not prone to spontaneous tumors.

In vitro analyses in cell lines (ES cells and MEFs) showed a strong phenotype. In homozygous and hemizygous conditions, the mutant cells are deficient in recruitment of RAD51 after DNA damage. This leads to increased genomic instability and corresponds to their slower growth rates. However, the meiotic progression in these mutant mice is quite normal. Spermatocyte analyses showed normal RAD51 foci at the leptotene/zygotene stages and clearance of YH2AX (DNA damage marker) foci in the late pachytene stage. This suggests that the meiotic recombination in BRCA2L2510P mutants is unhampered. This may be possible as during meiosis the homologous chromosomes are already paired, which may bypass the BRCA2-DSS1 interaction for removal of RPA nucleofilaments leading to RAD51 loading. To verify this hypothesis, we generated S-phase specific (where the homologous DNA is nearby) DNA double strand breaks using low dose camptothecin and did the RAD51 loading assay. Surprisingly, now we could observe normal RAD51 loading pattern in the mutants as it is in the wild type MEFs.

Conclusion- Our results indicate differential requirement of BRCA2-DSS1 interaction for RAD51 loading and this interaction is dispensable during S-phase and meiosis. This may the reason why the mutant mice are fertile even though the lack normal RAD51 loading efficiency in somatic cells.

Apoptosis induced nuclear bursting in tumor cells drives S100A4 mediated metastatic outgrowth

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Metastasis is a process marked by massive amounts of cell death, with only the fittest tumor cells surviving to colonize distant organs. There are different forms of cell death with distinct morphological, molecular and genetic features, significantly affecting cancer cell expansion, therapeutic resistance and evasion of immune surveillance. Apoptosis, which accounts for the majority of homeostatic cellular turnover, can be harnessed by tumor cells to enhance metastatic functions [3]. High apoptotic indexes have been correlated with malignancy of glioblastoma and non-small-cell lung cancer [4-6]. Here we present evidence that apoptosis in tumor cells triggers calcium mediated activation of Padi4 leading to the citrullination of chromatin and subsequent bursting of the nucleus, a process we have named exsporosis, which defines nuclear bursting broadly irrespective of cell type or treatment condition. Exsporosis results in an extracellular DNA/protein complex, herein exsporosi, and using cell based and animal assays, we demonstrate that exsporosi are found in metastatic lesions and they enhance metastatic outgrowth of breast cancer in the lungs. Mechanistically, S100A4, which we identified as highly abundant in exsporosi, mediates metastatic outgrowth via binding to the RAGE receptor of tumor cells leading to ERK activation. S100A4 utilizes exsporosis for not only its secretion into extracellular spaces but its function by binding to chromatin in exsporosi. Furthermore, the lung environment enhances Padi4 expression in tumor cells thus potentiating exsporosis. Consistent with this, we show that Padi4 correlates with lung metastasis in basal breast cancer.

Collectively, our study demonstrates a novel mechanism of how tumor cells utilize apoptosis to overcome unfavorable conditions during metastasis, especially micro- to macro-metastasis. In a clinical setting, we also highlight the importance of understanding exsporosis in tumor cells as treatments inducing apoptosis in tumor cells can lead to rapid and uncontrollable exsporosis with untold effects on relapse, resistance and therapy induced metastasis.

A Novel Dendritic Cell Population Infiltrates Murine Breast Cancer Tumors along with Convectional Dendritic Cells, Enhancing the Immune Response after Heterodimeric IL-15 (hetIL-15) Monotherapy

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Background and Hypothesis: IL-15 is a cytokine inducing the proliferation and cytotoxic function of lymphocytes, including CD8+ T and NKs cells and has anti-tumoral activity in many preclinical models. We have produced a native heterodimeric form of IL-15 (hetIL-15), which has advanced in clinical trials. The objective of this study was to further understand hetIL-15 function in tumors and especially hetIL-15 mediated interactions between lymphoid and myeloid cell populations in the tumor microenvironment of different murine models of breast cancer.

Study Design and Methods: We studied the therapeutic efficacy of hetIL-15 administration in two orthotopic mouse breast cancer models, 4T1 and EO771 respectively. hetIL-15 effects on tumor infiltrating immune cells were evaluated by flow cytometry and immunohistochemistry in both models and using transcriptomics and single cell RNA sequencing for the EO771 model.

Results and Conclusions: hetIL-15 treatment showed a profound tumor growth delay on the EO771 breast cancer model, resulting in complete tumor regression in ~40% of the treated mice, which also led to long term immunological protection against subsequent tumor rechallenges. Moreover, hetIL-15 also caused a significant tumor delay in 4T1 model. Despite the well described effects of hetIL-15 on accumulation and activation of cytotoxic effector cells, our transcriptomic analysis revealed additional enriched gene signatures in the treated tumors. These signatures were related to interactions between lymphoid and myeloid cells, T cell migration and antigen presentation. Moreover, flow analysis showed a significant increase of infiltrating conventional type 1 dendritic cells (cDC1s) mainly in EO771 breast tumors. 4T1 breast tumors showed increased infiltrating conventional type 2 dendritic cells (cDC2s). Importantly, our flow cytometry analysis revealed an additional novel distinct DC population characterized by CD103intCD11b+ immunophenotype, which was mostly evident in tumor tissues treated with hetIL-15 in both models. Phenotypic profiling of this novel DC population identified expression of several cDC1 specific markers, including CD103, IRF8 and XCR1. Single cell RNA seq analysis on sorted tumor infiltrated CD11c+ positive cells verified our initial findings, that identify a distinct DC population with unique gene signature, which is different from the conventional DCs, has antigen cross-presenting abilities and is abundant only in the treated tumors. It is noteworthy to point out, that both cDC1s and the novel DC population were inversely correlated with the tumor size in the EO771 model. In conclusion, hetIL-15 enhances the interplay between cytotoxic effector cells and antigen presenting cells, promoting a strong and long-lasting anti-cancer immune response. This response is further strengthened by hetIL-15 mediated tumor infiltration of a novel distinct dendritic cell population.

Relevance and Importance: These findings suggest that hetIL-15 affects not only the cytotoxic effector cells but also increases the tumor infiltration of dendritic cells, enhancing the interactions between these cell populations, and thus generating a strong immune response with long term immunological protection. Our results further suggest that hetIL-15 is a promising therapeutic agent in treatment of breast cancer.

Genetics, Genomics, Chromatin, Signal Transduction and Transcription

FGF receptor 1 and 2 signalling in the somites is essential for the morphogenesis of the muscles, bones and tendons of the trunk and abdomen

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Background and Hypotheses

Fibroblast growth factor (FGF) signalling through FGF receptors (FGFR) 1 and 2 is well known to be implicated in cancer biology, with increased FGF signalling promoting cellular proliferation, survival, differentiation, migration and epithelial-to-mesenchymal transition (EMT). FGFR1/2 signalling is also needed for the morphogenesis of many different tissues during embryonic development. FGFR1/2 activation in the pre-somitic mesoderm is needed to form the somites, the progenitors of the muscles of the trunk, back and abdomen. The somites also give rise to the skeleton and tendons of the abdomen and trunk. There is no published genetic data investigating whether FGFR1/2 signalling within the somites themselves is needed to form these downstream tissues. We will use mouse genetic models to ask whether FGFR1/2 signalling has any function in the somites.

Study Design and Methods

We utilised Cre-loxP technology (the Meox1Cre line) to conditionally delete Fgfr1 and Fgfr2 in the somites shortly after they had formed. Double heterozygous embryos were used as controls and double mutants as experimental samples.

Results and Conclusions

Examining the skeletons of perinatal embryos revealed that the ribs and neural arches were misshapen or absent, consistent with defects early in their development. We also saw evidence of muscle defects. We next studied the expression of genes needed for skeleton (Sox9), muscle (myogenin) and tendon (scleraxis) formation midgestation. Sox9 and scleraxis were strongly downregulated in the ribs and tendons, indicative of a loss of tissue identity. The pattern of myogenin suggested that the muscle progenitors in mutants have not migrated as far or as coherently compared to controls, but their tissue identity is intact. Muscle formation requires EMT in the somites, and in the chick this has been reported to be induced by FGF activity. When we examined the expression of the EMT genes Snail1 and Zeb1 in the muscle progenitors, we unexpectedly found that their expression was upregulated. This is despite the loss of FGF activity in the somites, as measured by the strong downregulation of the FGF responsive genes Etv4, Etv5 and Spry4. Future work will resolve when the change in EMT occurs and how it affects the morphogenesis of the muscle progenitors. This project is still at an early stage, but our results thus far allow us to conclude that FGFR1/2 signalling is essential in the somites to form downstream tissues.

Relevance and Importance

This project will shed light on the role of FGF signalling in the somites. Previous studies have implicated several FGF ligands as well as FGFR4 in forming the muscles, bones and tendons. However, much of this work has been in chick embryos or has addressed the role of FGF signalling indirectly. As such, direct evidence in a mammalian model is sorely lacking. This research will allow us to integrate FGF signalling into signalling pathway models of muscle, cartilage and tendon formation, with potential relevance for tissue engineering and regenerative medicine. This project may also provide insights into the role of FGFR1/2 signalling in rhabdomyosarcoma or other sarcomas.

EWS-ETS fusion oncoproteins suppress the expression of transcriptional regulators of cell lineage or differentiation

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In most solid malignancies, an essential determinant of the plasticity required for metastasis is the genetic heterogeneity of the primary tumor that reflects an increasing mutational burden over time. However, in Ewing sarcoma (EWS), an aggressive bone and soft tissue sarcoma affecting children and young adults, there is a single oncogenic mutation that drives tumor development and must also regulate metastasis. The primary oncogenic event in most cases of EWS involves the EWSR1 locus and one of two members of the ETS family of transcription factors, FLI1 or ERG. The resulting fusion oncogenes (EWS-FLI1 or EWS-ERG) express aberrant transcription factors, collectively referred to as EWS-ETS that can function as activators and repressors of gene expression. Most studies of the EWS-ETS oncoproteins have focused on their activation of genes that favor cell proliferation, but recent studies have highlighted that repressed gene targets may be responsible for the metastatic phenotype of EWS cells. Here, we aim to identify gene regulatory networks that elevated levels of EWS-ETS suppresses but which become activated when EWS-ETS levels are lower, with a focus on transcription factors involved in defining cell lineage or differentiation.

To identify genes repressed by EWS-ETS fusion oncoproteins, we first depleted EWS-FLI1 via RNAi in two EWS cell lines, TC-32 and TC-71, and performed RNA-seq analysis of the EWS-FLI1-silenced cells versus control cells (siNeg-transfected). Using the criteria of a ¬±1.5-fold change, FDR of <0.05, and a minimum median of four transcripts per million (TPM) in EWS-FLI1-silenced cells, we categorized 1325 genes as exhibiting an increased expression in both cell lines. To identify transcription factors within this gene set, we focused on a curated set of proteins with bona fide DNA binding activity and identified 114 that showed significant increases in expression following depletion of EWS-FLI1. Using published epigenetic marks for EWS cells and relative expression of these genes in EWS tumors and cell lines, we selected 23 genes for further analysis. First, we assessed the regulation of these 23 genes by EWS-ETS in two further EWS cell lines, silencing EWS-FLI1 in SK-N-MC cells and EWS-ERG in ES5838. Using qPCR, we observed a significant increase in the expression of at least half of the 23 transcription factor genes in both cell lines. Based on published epigenetic marks for EWS cells, we designed loci-specific ChIP-PCR assays to assess the H3K27ac status of these 23 genes in SK-N-MC cells in the presence and absence of EWS-FLI1. We observed changes consistent with activation of gene expression (enrichment of the H3K27ac mark) at 22 of the 23 genes following the silencing of EWS-FLI1. Based on these findings, we are currently generating a panel of TC32 EWS cell lines in which we have overexpressed each of our first genes of interest, specifically, ETS1, SNAI2, JUNB, GLI2, RUNX2, EPAS1, or EGR1. Following confirmation of the ectopic-expression of each gene, we will subject the modified cells to RNA-seq and compare their expression profiles to those of control cells, and assess their phenotypes under different growth conditions, including as monolayers/multicellular spheroids.

Oncogenic LncRNAs drive histone variant mislocalization in cancer cells

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Chromosomal instability is a major defining event of cancer progression. The histone H3 variant CENP-A (Centromere protein A) is normally restricted to centromeres where it plays a fundamental role in centromere structure, identity and function. However, in a variety of tumors, we have reported that CENP-A can hijack the H3.3 chaperone pathways to deposit ectopically, thus invading regions such as the chr8q24 locus. Other than the presence of the proto-oncogene MYC, this region is typically a gene desert; however, the existence of a large DNase I hypersensitive site has driven us to examine non-coding transcription from this locus. Intriguingly, several non-coding RNAs are transcribed from the chr8q24 locus, making this region an oasis for non-coding transcription.

Long non-coding RNAs (lncRNAs) have less or no protein-coding potential, are over 200 nt in length with diverse cellular functions ranging from genome organization to transcription regulation. Deregulation of lncRNAs is one of the potential hallmark features of cancer progression. Overexpression of chr8q24 locus derived lncRNAs is frequently reported in many cancers and correlates with poor therapeutic outcome and recurrence. Here, we hypothesize that chr8q24 derived oncogenic lncRNAs are unwitting players in altering the local chromatin landscape by recruiting incorrect chaperone-histone variant complexes.

We knocked down the top candidate lncRNAs at chr8q24 locus (PCAT1, PCAT2, CCAT1, CCAT2, and PVT1), to study ectopic CENP-A (eCENP-A) localization in metastatic SW480 colon cancer cells. Interestingly, using coIF-DNA-FISH, we found that disruption of chr8q24 lncRNAs significantly reduced eCENP-A. Releasing the cells from the knockdown treatment significantly rescued the eCENP-A level at this locus. Remarkably, knocking down H3.3 chaperones (HIRA and DAXX) following the lncRNA knockdown prevented the cells from acquiring eCENP-A at the chr8q24 locus. Levels of the CENP-A at centromeres were not affected by the chr8q24 lncRNA perturbation, which confirms that these non-coding transcripts specifically alter the local chromatin from where they were transcribed. Furthermore, we find that the colocalization of kinetochore proteins with eCENP-A at chr8q24 impacts the chromosomal architecture, resulting in an increasing in chromosomal break within this region. These data suggest a novel epigenetic mechanism linking locus and cancer-specific lncRNAs to aberrant chromatin structures in cancer cells.

Elucidating Functional Enhancers Governing Pancreas Cell Identity

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Background
The human pancreas is a complex organ featuring heterogeneous cellular composition: the endocrine pancreas consists of hormone producing alpha, beta, and delta cells, while the exocrine pancreas contains acinar and duct cells that aid with digestion. Dysfunction of pancreatic cells results in diseases like pancreatitis, pancreatic cancer, and diabetes mellitus. Genomic enhancers,Äî short, noncoding DNA sequences, are principle regulators of gene expression, cell identity and function. In addition, the majority of disease linked DNA variants reside in these enhancer regions. Thus, having a comprehensive understanding of the enhancer landscape and function in pancreas cells would accelerate discoveries of new therapeutic targets for pancreatic disorders. However, the mechanism linking these enhancer regions to cell-type specific gene regulation, particularly in endocrine cells, remains unclear. Here, we have combined cell sorting of primary pancreatic cells with both Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq) and Hi-C linked with chromatin immunoprecipitation (HiChIP) experiments to delineate the physical and functional interactions between enhancers and their target genes.

Study Design and Methods
We used a flow cytometry based method to purify primary cells from the human pancreas. These cells were used in ATAC-seq and HiChIP experiments to identify potential looping interactions between enhancer regions and gene transcription start sites. Using computational analyses, we characterized significant ATAC-seq peaks and HiChIP interactions specific to certain cell types. We also performed HiChIP in EndoC-BH1 cells, an insulin-producing human beta cell line, for proof-of-concept experiments establishing a CRISPR-interference and -activation system in the EndoC-ÔÅ¢H1 cells, used to determine the functionality of these putative enhancers.

Results
We identified a total of over 129,000 putative cell-specific enhancer regions for alpha, beta, delta, acinar, and duct cells. To our knowledge, this is the first known dataset featuring regions for all five pancreatic cell types. We integrated this ATAC-seq data with HiChIP interactions for each cell type to reveal thousands of enhancer-promoter interactions along with their associated genes, with a focus on islet beta-cells due to their central role in diabetes. Preliminary results identified unique enhancer-promoter interaction loops in EndoC-BH1 cells, whose anchors correspond to beta-cell specific accessible chromatin regions. To test whether these looping and accessible chromatin regions can control transcription, we systematically targeted several regions around the PCSK1 gene using the CRISPRi (KRAB) and CRISPRa (VP64) systems. We found that perturbation or activation of enhancers that loop to the PCSK1 promoter showed significantly decreased or increased levels of transcription respectively. Meanwhile, targeting regions with no loops had no effect.

Relevance and Importance
Our data will provide critical knowledge on the underlying gene regulatory mechanisms that govern pancreatic cell identity and function to ultimately advance therapies for pancreatic disorders.

Immunology, Virology, and Metabolism

GPC3 is a potent target for liver cancer immunotherapy using engineered, nanobody-based CAR T cells

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Background and Hypotheses
Glypican 3 (GPC3), a cell surface molecule associated with early development is an established biomarker of hepatocellular carcinoma (HCC). GPC3 has been shown to be a key player in the Wnt signaling cascade and upregulation of Wnt signaling is a major factor in HCC pathogenesis; thus, disruption of the GPC3/Wnt interaction is an attractive therapeutic strategy. In a vast compendium of work, HN3, a human GPC3 nanobody (VH) isolated from a phage library, was shown to abrogate HCC tumors and block the distally located Wnt binding domain of GPC3. Here, we show how rationally designed, engineered Chimeric Antigen Receptor (CAR) T cells, demonstrate improved binding capacity and enhanced CAR T activity.

Study Design and Methods
Incorporation of HN3, into multiple CAR formats yielded 4 different
constructs, which were screened for antigen-specific and antigen-independent signaling using a luciferase reporter system. The HN3 CAR was engineered to include IgG4-derived short, medium and long hinges and Fc regions to examine how the length and structure of each hinge might affect reach to the GPC3 epitope of interest and induce cell killing. We hypothesized that optimization of the spacer domain may enhance T cell activation. We used a luciferase-based cell killing assay to test CAR efficacy in HCC cell lines and the top candidates were leveraged for in vivo studies.

Results and Conclusions
We showed that HN3 containing the IgG4 short hinge displayed the highest cell killing in Hep3B, HepG2, and Huh7 cell lines. In contrast to the CD8 hinge, used in the original HN3 CAR construct, the IgG4 short hinge markedly improved HN3 cell killing activity by 30-40% in low (2:1) and high (25:1) effector to target ratios. In GPC3 knockout cells in the Hep3B background, engineered HN3 CAR T cells showed no activity which means that cell killing is antigen specific. We tested the in vivo activity of these rationally, designed engineered CAR T cells in an NSG mouse model and showed that the HN3 short hinge CAR T cell regressed and eliminated HCC tumors within 7 days. The engineered CAR T cells harbored an overwhelming number of CAR positive CD8 T cells. The T cells displayed both memory and effector function in vivo. Structural models which quantify disorder in protein-protein interactions validate these findings and suggest that the IgG4-short hinge stabilized the HN3 nanobody presumably forming a better immune synapse with the target on tumor cells.

Relevance and Importance
HCC, the most common form of liver cancer, affects 750,000 – 1 million people worldwide each year and is projected to increase, in contrast to most other solid cancers. The highest burden of mortality is seen in East Asia and sub-Saharan Africa, where medical resources are limited. HCC recurrence rates are very high, and the 5-year survival rate remains below 12%. Thus, it is critical to pursue all possible therapeutic modalities. Here, we show that engineered nanobody-based HN3 CAR T cell therapy shows potent efficacy for treatment of aggressive HCC enabled by Wnt dysregulation.

Oncogenic human polyomaviruses interact with host chromatin during native virus infection and after integration in cancer

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Background and Hypotheses

Human polyomaviruses predominantly exist as lifelong latent subclinical infections in the majority of the population and cause disease mainly in immunocompromised individuals. Specifically, Merkel cell polyomavirus (MCPyV) is the primary cause of Merkel cell carcinoma (MCC) and BK polyomavirus (BKPyV) has been linked to the development of bladder cancer in kidney transplant recipients. Recently, it has been demonstrated that MCPyV preferentially integrates into areas of open chromatin through microhomology-mediated end joining, frequently coinciding with focal amplifications of the host genome. Our lab has also found evidence of similar integration-amplification patterns in BKPyV-associated bladder cancer. CCCTC-binding factor (CTCF), when paired with cohesin, controls chromatin architecture and subsequently gene expression through the formation of chromatin loops through DNA strand extrusion. The anchor sites of these loops are vulnerable to DSBs during replication stress and both the MCPyV and BKPyV genomes contain predicted CTCF binding sequences, indicating a potential role for CTCF binding in the viral life cycle. Furthermore, polyomavirus integration events are frequently observed in close proximity to host CTCF insulator sequences. We hypothesize that CTCF-mediated host-virus chromatin interactions are necessary for polyomavirus gene transcription and genome replication during normal infection and contribute to accidental integration and host-cell transformation events in polyomavirus-associated cancers.

Study Design and Methods

Native BKPyV and MCPyV infections were performed in normal-like urothelial cells and primary dermal fibroblasts, respectively, and chromatin was collected after viral genome uncoating, approximately 18-24 hours post infection. To assess whether CTCF binds polyomavirus genomes, chromatin immunoprecipitation sequencing (ChIP-seq) was used to unbiasedly identify CTCF binding sites in the host and viral genomes after infection. We subsequently used 4C-seq, a sequencing technology that reveals the sequences of physically close chromatin through the digestion and ligation of crosslinked DNA, to identify the viral genome sequences in contact with host chromatin during infection and in integrated virus in MCC.

Results and Conclusions

Within the polyomavirus genomes, we identified CTCF binding sites predominantly flanking the Large T antigen gene and within the late coding region by ChIP-seq. Through 4C-seq, we identified recurrent viral contact sites across the human genome for both BKPyV and MCPyV in normal cell infections and in MCC. Our results not only suggest an enrichment of contact sites at CTCF-cohesin anchors but also enrichment near cell lineage defining transcription factors, which may promote expression of viral genes. Additional knockdown and mutagenesis studies will be performed to identify the functional importance of these interactions.

Relevance and Importance

This study identifies that polyomavirus DNA is bound by CTCF and interacts with host chromatin in normal cells and in polyomavirus-mediated cancer. Studies in other viral families suggest that, these interactions are potentially important for latent and productive infection, as well as for carcinogenic reprogramming of the host cell. Further experiments will also address whether these interactions promote viral genome integration and the mechanisms by which they contribute to cellular transformation.

Differential replication of BK polyomavirus strains in normal and malignant urothelium

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Background and Hypothesis

BK polyomavirus (BKPyV) is ubiquitous in humans, with most children acquiring the virus before the age of 10 and >80% of all adults being seropositive for BKPyV. BKPyV establishes a lifelong subclinical infection in the urinary tract and often reactivates in immunosuppressed individuals, such as transplant recipients, causing severe disease. Additionally, solid organ transplant recipients with a previous history of BKPyV viremia or nephropathy are at an increased incidence of bladder cancer with over 20% harboring integrated BKPyV. This is in stark contrast to the rate of BKPyV positivity in bladder cancer of immunocompetent individuals, which is less than 5%, with integration being even rarer. To better understand the relationship between BKPyV infection and bladder cancer biology, we investigated the dynamics of infection between existing bladder cancer cell lines and different strains of BKPyV.

Study Design and Methods

We evaluated the binding, entry, and replication of archetype BKPyV found in healthy individuals and the more pathogenic, rearranged BKPyV found in disease on five bladder cancer cells lines and one normal-like immortalized bladder epithelial cell line. We utilized fluorescently labeled pseudovirus and high-throughput imaging to determine binding efficiency, pseudovirus delivery of a GFP expression construct and flow cytometry to assess viral entry, and native virus infection followed by qPCR to evaluate BKPyV genome replication. Lastly, we performed RNA sequencing on cells infected with rearranged and archetype BKPyV at 1, 3 ,5, and 7 days post infection to identify genes and pathways responsible for observed differences in infection phenotypes.

Results and Conclusions

Consistently, non-muscle-invasive bladder cancer cell lines readily replicated both archetype and rearranged viruses while muscle-invasive bladder cancer cells lines showed little to no replication for either strain. However, there were no significant differences in entry and binding between any cell lines. Differential gene expression of the RNA sequencing data identified candidate genes and pathways predominantly associated with the cell cycle that explain the observed differences in viral replication for further investigation. Our results suggest that somatic alterations during different stages of bladder carcinogenesis affect key pathways involved in the replication of BKPyV and impact how the virus interacts with cancer cells versus normal cells.

Relevance and Importance

While there has been growing evidence for a role of BKPyV infection in the development and outcome of bladder cancer in solid organ transplant recipients little is known about how this ubiquitous urinary pathogen interact with cancer cells from immunocompetent individuals. Our results indicate that there are molecular differences between non-muscle invasive and muscle invasive bladder cancer that impact viral replication after entry into the cells. Continued interrogation of BKPyV infection in bladder derived cell lines may shed light on how this virus impacts bladder cancer biology and patient outcome in both immunocompromised and immunocompetent individuals.

CAR T cells targeting GPC2 as a treatment for childhood cancer

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Background and Hypothesis

Glypican 2 (GPC2) is a cell-surface heparan sulfate proteoglycan that is a new therapeutic target for childhood cancers. Though GPC2 has little to no expression in healthy tissues, it is expressed at high levels in several childhood cancers, including neuroblastoma. Its highly tumor-specific expression makes GPC2 a promising therapeutic target, as GPC2-targeted therapies may avoid on-target, off-tumor effects. Additionally, GPC2 may function in neuroblastoma cell proliferation, by acting as a co-receptor in the canonical Wnt signaling pathway to upregulate transcription of the oncogene MYCN, which is consistently correlated with poor clinical outcome in neuroblastoma. Thus, in addition to being tumor-specific, antibody-based therapeutics targeting GPC2 may also block GPC2‚Äôs action in Wnt signaling, making them more effective.

Study Design and Methods

Our lab has isolated a high-affinity mouse monoclonal anti-GPC2 antibody (CT3) and constructed chimeric antigen receptor (CAR) using CT3 single-chain variable fragment (scFv). We tested the cytotoxicity of the CT3 CAR T cells against GPC2-positive and GPC2-negative neuroblastoma cell lines in an in vitro cell killing assay. We studied the ability of the CT3 CAR T cells to regress neuroblastoma tumor burden in mouse in metastatic and orthotopic neuroblastoma mouse models. Finally, in an attempt to reduce the immunogenicity of the mouse antibody, we humanized CT3 by complementary determining region grafting using human frameworks and constructed CARs from each of them, which we evaluated in vitro and in vivo.

Results and Conclusions

All five GPC2-targeted GPC2 CAR T cells potently and specifically kill GPC2-positive neuroblastoma. Thus far, the CT3 CAR has been shown to regress neuroblastoma tumor burden in mice, with the mice remaining tumor-free for up to six weeks after CAR T cell treatment. Our preclinical testing of GPC2-targeted CAR T cells has validated GPC2 as a therapeutic target in neuroblastoma.

Relevance and Importance

CAR T cells have yet to be widely translated to solid tumors. In the case of neuroblastoma, the standard of care is intensive multimodal treatment and the disease remains fatal in 45% of patients. Thus, new, effective therapies for neuroblastoma, like CAR T cells, would feel a great need. Additionally, GPC2-targeted developed for neuroblastoma have the potential to be translated to several other childhood cancers that have similarly high GPC2 expression.

Molecular and Cellular Biology and Microbiology

Investigating the molecular and cellular roles of metabolism on RNA epitranscriptomics in renal cell carcinoma

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Background and Hypothesis
In cancer, tumorigenesis is dependent upon reprogramming cellular metabolism. In a number of cancers, mutations to enzymes in the tricarboxylic acid (TCA) cycle can lead to the accumulation of 2-hydroxyglutarate, fumarate or succinate. Accumulation of these metabolites has been shown to inhibit enzymes in the aKG-dependent dioxygenase family, which includes a number of DNA and histone demethylases. Recently, the RNA demethylases FTO and ALKBH5 were shown to be members of the aKG-dependent family. The RNA modification N6-methyladenosine (m6A) is the most abundant internal modification in mRNA and is thought to influence all aspects of mRNA metabolism, including splicing, stability, and translation. The central hypothesis is that metabolic rewiring leads to the disruption of RNA demethylation, thereby promoting aberrant mRNA processing, leading to altered gene expression programs and driving cancer.

Study Design and Methods
Our study utilizes hereditary leiomyomatosis and renal cell carcinoma (HLRCC) as a model system to assess alterations in RNA methylation and RNA processing events in response to metabolic rewiring. Cells derived from patient tumors with inactivating mutations to the TCA enzymes succinate dehydrogenase (SDH) or fumarate hydratase (FH) are compared to cells where enzymatic activity has been restored. Global changes in both RNA modifications and TCA cycle intermediates are determined by tandem mass spectrometry. We utilize several next-generation sequencing approaches to assess m6A changes at the transcript level (m6A-IP), mRNA splicing (RNA-Seq and Nanopore native RNA sequencing), mRNA half-life (SLAM-Seq), and mRNA translation (ribosome profiling). To assess the role of RNA methylation in cancer-like phenotypes, we use CRISPR-Cas9 to disrupt the expression of m6A writers or erasers in several HLRCC cell lines and measure changes through wound-healing and invasion assays.

Results and Conclusions
In cells with loss of FH activity, we observe an increase in both the global and transcript-specific levels of m6A. Analysis of mRNA half-life reveals shorter mRNA half-life in cells with fumarate accumulation, supporting the hypothesis that fumarate accumulation disrupts RNA processing. To assess the role of RNA methylation in tumorigenesis, we used CRISPR-Cas9 to disrupt the expression of m6A writers or erasers in several cell lines. Our current work focuses on the use of these knockout cell lines to assess the role of m6A-dependent pathways in tumorigenic phenotypes, including cellular proliferation, migration, and invasion while future work will focus on testing targets of interest in a mouse xenograph model.

Relevance and Importance
Eukaryotic cells use multiple mechanisms to regulate geneticprograms. Epitranscriptome dysregulation has been implicated in multiple cancers, including acute myeloid leukemia, glioblastoma, lung cancer, and kidney cancer. The impact of fumarate accumulation on the RNA epitranscriptome remains an unexplored area. This work will increase our understanding of the mechanisms by which metabolism and RNA modifications interact to influence gene expression programs in cancer and may provide targets for the development of novel clinical treatments of patients with cancers where epitranscriptomic dysregulation occurs.

Substrate specificity of the yeast Hsp70 cochaperone, Ydj1, reflects multiple substrate binding sites

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Molecular chaperones are important components of the cellular machinery involved in maintaining protein homeostasis. The Hsp70 family of molecular chaperones functions in nearly every cellular process by promoting ATP-dependent protein folding, remodeling, and activation. The activity of Hsp70 is regulated by two cochaperones, a J-domain protein (JDP) and a nucleotide exchange factor. The ability of Hsp70 to act on a wide variety of substrates is facilitated by collaboration with multiple JDP partners with diverse structures and cellular localizations. The mechanism of substrate binding by JDPs is an important component of this system that has yet to be completely understood.

Our working hypothesis is that JDPs require multiple substrate binding sites in order to act on a wide variety of substrates. To better understand substrate binding by JDPs, we examined the interaction of a S. cerevisiae cytosolic JDP, Ydj1, with various substrates. Ydj1 and its homologs are comprised of a J-domain, a G/F rich region, a Zn binding domain, two structurally similar C-terminal domains, CTD I and CTD II, and a C-terminal dimerization domain. We generated substitution mutants in Ydj1 with the objective of disrupting JDP-substrate interactions and identifying specific interaction sites. We targeted residues near a known peptide binding region in CTD I, and in the structurally homologous region of CTD II. First, we tested the ability of Ydj1 wild-type and mutants to bind a fluorescently labelled peptide previously co-crystallized with Ydj1 (Li et al. 2003). While wild-type Ydj1 bound the peptide as expected, several mutants showed defects in binding to the peptide. Next, we tested the ability of Ydj1 mutants to bind and prevent the aggregation of chemically denatured luciferase and observed that a few mutants in both CTD I and CTD II were defective in the ability to prevent luciferase aggregation. Last, using pull-down assays we tested the ability of these Ydj1 mutants to bind to native protein substrates. Compared to wild-type, several mutants showed altered binding to the native protein substrates tested. In addition to substrate binding, we assessed the ability of Ydj1 mutants to collaborate with yeast Hsp70, Ssa1, for protein reactivation and observed that a few Ydj1 mutants were defective in their ability to collaborate with Hsp70 in protein remodeling. Taken together, the results suggest that Ydj1 binds substrates through multiple binding sites in both CTD I and CTD II. The existence of multiple substrate binding sites in a JDP may be one mechanism of increasing the number and diversity of substrates for Hsp70 action, shedding light onto the role of JDPs in the Hsp70 chaperone system. Overall, understanding the mechanism of Ydj1 and chaperones in general is essential to provide the foundation for discovering preventions and treatments for diseases involving protein misfolding, including cancer and neurodegenerative diseases.

A live-cell biosensor for SCFÔÅ¢TrCP reveals expression vs. activity dependency of transformed cells

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SCF E3 ubiquitin ligases are a family of proteins that promote the proteasome-mediated degradation of almost 80% of cellular proteins and play a crucial role in cellular physiology, signaling, and transformation. SCFÔÅ¢TrCP is a particularly important member of this family, as it plays an important regulatory role inflammation (IKBa), apoptosis (BimEL and Pdcd4), Wnt signaling (Œ≤-Catenin), cell cycle regulation (Emi1, Wee1, and CDC25) and many more. Furthermore, SCFÔÅ¢TrCP is often deregulated in many types of cancers, and recent studies have indicated that inhibiting SCFÔÅ¢TrCP can sensitize some cancer cells to chemotherapy. While SCFÔÅ¢TrCP has been characterized extensively using in vitro biochemical approaches, little is known about how SCFÔÅ¢TrCP is regulated in both normal and transformed cells, and there are still no specific SCFÔÅ¢TrCP inhibitors available. Therefore, we developed a fluorescent biosensor that allows us to quantitatively measure SCFÔÅ¢TrCP activity in thousands of individual cells in real-time. We used live-cell microscopy and an automated single-cell tracking and image analysis pipeline, to watch how SCFÔÅ¢TrCP activity changed over time in response to various stimuli and perturbations. Strikingly, we found that SCFÔÅ¢TrCP is a relatively weak ubiquitin ligase that is mostly incapable of completely degrading its substrates. Instead, SCFÔÅ¢TrCP appears to remain constitutively active throughout the cell cycle and functions to maintain discreet steady-state levels of its substrates. Furthermore, consistent with published reports, we found that SCFÔÅ¢TrCP activity is elevated in a diverse set of cancer cell lines. However, we found no correlation between the expression levels of ÔÅ¢TrCP and SCFÔÅ¢TrCP activity. Importantly, we found that SCFÔÅ¢TrCP activity as measured by the biosensor is a better predictor of chemosensitivity for different therapeutic drugs. In the future, we plan to use our SCFÔÅ¢TrCP sensor to develop a screening platform that can be used to screen small molecules that can target SCFÔÅ¢TrCP activity in transformed cells. In addition to helping to identify new lead-compounds for the inhibition of SCFÔÅ¢TrCP, this unique approach may serve as a proof of concept to develop screening platform for other E3 ubiquitin ligases as well.

Substrate binding by DnaJ, a cochaperone of the E. coli Hsp70 homolog, DnaK

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Molecular chaperones play an important role in maintaining proteostasis in cells by aiding in the folding of nascent polypeptides, preventing aggregation of unfolded or non-native proteins, and mediating refolding and reactivation of misfolded proteins. Hsp70 is a ubiquitous molecular chaperone that promotes protein remodeling by coupling ATP hydrolysis to the binding and release of substrates. Two cochaperones, a J-domain protein (JDP) and a nucleotide exchange factor, function with Hsp70 in this system. Hsp70s are able to act on a multitude of substrates by partnering with a wide range of JDPs of diverse structure and function. The role of JDPs in the Hsp70 chaperone system is important, though the mechanism of action is not fully understood. Our hypothesis is that JDPs can interact with a variety of substrates by utilizing multiple substrate binding sites. In this study we have explored the ability of an E. coli JDP, DnaJ, to bind and target substrates for protein remodeling by Hsp70. DnaJ is comprised of a J-domain followed by a glycine and phenylalanine rich region, two structurally homologous C-terminal domains (CTD I and CTD II), and a dimerization domain. A zinc-binding domain lies embedded in CTD I as well, classifying DnaJ as a type I JDP. Structural studies of two yeast JDPs demonstrated that a peptide binding site exists in CTD I and recent work showed that CTD I and CTD II both have sites that bind unfolded substrates.

To study substrate binding by DnaJ, we constructed substitution mutants in CTD I and CTD II of DnaJ. The mutant proteins were compared to wild-type DnaJ for the ability to bind non-native substrates, native substrates and peptides. The DnaJ mutants were also compared to wild-type DnaJ for the ability to reactivate denatured proteins in combination with DnaK and the nucleotide exchange factor GrpE. Our results showed that some of the DnaJ mutants in both CTD I and CTD II were impaired in substrate binding. Importantly, differences in the ability to bind specific substrates as well as peptides were observed, suggesting DnaJ possesses substrate specificity. In addition, DnaJ mutants showed defects in reactivation of denatured proteins in collaboration with DnaK compared to wild-type DnaJ, showing that the sites identified are important for the function of DnaJ in protein reactivation in collaboration with DnaK. Together, these results indicate that JDPs have multiple sites that are important for binding substrates and that the sites exhibit substrate specificity.

Since protein misfolding and aggregation contribute to a large number of human diseases, including cancer, Alzheimer‚Äôs, Parkinson‚Äôs, type II diabetes, cystic fibrosis, and prion diseases, our work to elucidate how Hsp70 and JDPs function is important and will help pave the way for research in prevention and treatment of diseases that involve misfolded and aggregated proteins.

Poster Presentations

Bioinformatics, Epidemiology and Translational Research

The Landscape of Precision Cancer Combination Therapy: A Single-Cell Perspective
Rahulsimham Vegesna[1], Saba Ahmadi[2^], Pattara Sukprasert[3^], Sanju Sinha[1], Fiorella Schischlik[1], Natalie Artzi[4,5,6], Samir Khuller[3], Alejandro A. Sch√§ffer[1*], Eytan Ruppin[1*]
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Genetics, Genomics, Chromatin, Signal Transduction and Transcription

Deconvolution of downstream signaling of the innate immune checkpoint CD206 in CD206high M2-like tumor-associated macrophages yields novel therapeutic opportunities
Sitanshu Singh[1] *, Rushikesh Sable[1] *, Emily Major[1], Bogdan Domrachev[1], Theresa Guerin[2], Serguei Kozlov[2], Bolormaa Baljinnyam[3], Udo Rudloff[1]
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Determination of the contribution of EWSR1 and EWS-FLI1 to the phenotype of Ewing sarcoma cells via a CRISPR-mediated tagging strategy of the endogenous proteins.
Soumya Sundara Rajan[1], Katelyn R. Ludwig[1], Javed Khan[1], Raj Chari[2], Natasha J. Caplen[1]
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Immunology, Virology and Metabolism

Intrinsic Requirement for Stat3 for Latency Establishment of Murine Gammaherpesvirus In Vivo
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hetIL-15 Monotherapy Increases the Intratumoral CD8+ T Cytotoxic Cells and Reverses the Metabolic Dysfunction, in Murine Breast Tumors.
Sevasti Karaliota[1], Dimitris Stellas[2], Vasiliki Stravokefalou[2,3], Bethany Nagy[2,4], Cristina Bergamaschi[4], Barbara K. Felber[4] and George N. Pavlakis[2].
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Toward Therapeutic Modulation of p53 Isoform Œî133p53Œ± in the Tumor-Immune Microenvironment
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Molecular and Cellular Biology and Microbiology

Finding novel regulatory elements of RNA modifying proteins in cancer metabolism.
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Investigation of the N-Myc and LDB1 Interactomes in Neuroblastoma Cells
Samuel Chen[1], Zhihui Liu[1], Jason Hong[1], Amanda Ciardiello[1], and Carol J. Thiele[1]

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Uncovering the Expression, Localization and Biosynthesis of the GalNAc-Tyrosine Posttranslational Modification

Molly D. Congdon[1], Sudipto Das[2], Crystal Li[1], Li Xia[1], Tiffany Bellomo[1], Ruslan Gibadullin[1], Thorkell Andresson[2], Jeffrey C. Gildersleeve[1]
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Characterizing the mechanism of degradation of the mitochondrial matrix temperature-sensitive protein yah1p^(ts)

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Elucidating the modification status of tRNAs in response to environmental and metabolic stress.

Mariana D. Mandler[1], Wilfried Guiblet[1], Christina M. Fitzsimmons[1], Kayla S. McDonald[1], Pedro J. Batista[1]
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Cell cycle dependence of p53 dynamics during the DNA damage response

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