NATIONAL® CANCER INSTITUTE

Center for Cancer Research

12th Annual

Fellows and Young Investigators Colloquium

March 26–27, 2012

Natcher Auditorium (Building 45)
NIH-Bethesda, Maryland

FOR INTERNAL USE ONLY

NCI Center for Cancer Research

Fellows & Young Investigators

Welcome to the 12th Annual CCR-FYI Colloquium!

On behalf of the National Cancer Institute Center for Cancer Research Fellows and Young Investigators (FYI) Association, it is a great pleasure to welcome you to the 12th Annual NCI CCR FYI Colloquium. FYI was organized to foster the professional development of all NCI CCR trainees. The colloquium is a significant component of the FYI association's activities that provides an exceptional opportunity to network with other fellows and distinguished keynote speakers, present research, participate in informative and skill-building workshops, and attend a diverse career fair.

We have an exceptional program for this year's colloquium! Our outstanding keynote speakers are Dr. Jeffrey Schlom, Dr. Natasha Caplen and Dr. Brigid Hogan. Joanna Rudnick, a cancer survivor, is an award-winning documentary filmmaker who will give an inspiring presentation about her experience with cancer and the impact it has on her life. There is something for everyone with workshops that range from traditional, informative panel discussions to interactive, skill building presentations. The Career Fair continually builds from previous years, and this year is no exception! Our vendors include NIH and NCI facilities that can help you attain your research goals, organizations that provide excellent volunteer community activities, and companies from industry currently looking to hire bright researchers. The colloquium also provides an excellent opportunity for fellows to present their work through poster and oral presentations, with a chance for winning a travel award.

The FYI Association continues to work with the NCI CCR Office of Training and Education (OTE) to enhance intramural training, promote communication and networking in the NCI community, serve as a liaison to administrative programs, and encourage community involvement.

We accomplish these goals by:

- **Promoting communication between mentors and trainees**
- **Organizing social events**
- **Providing opportunities for writing, presenting, and leadership**
- **Promoting on-campus and off-campus community involvement through our NEW Outreach Subcommittee**
- **Providing exceptional networking and skill-building opportunities through our annual Colloquium**
- **Evaluating the most current demographics and opinions of NCI CCR trainees with our annual survey**

We sincerely thank Dr. Robert Wiltrout, his administrative staff, Dr. Jonathan Wiest, and the OTE for continued support for the FYI Association. This is an exciting time for FYI as we continue to grow and partake in new tasks and activities that help further support both scientific training and quality of life as an NCI Fellow. Explore our other activities by visiting our website: http://ccr.ncifcrf.gov/careers/fellows/default.aspx, or by emailing us at nciccrfyi@mail.nih.gov.

On behalf of the FYI Association, we hope you take full advantage of what the colloquium has to offer!

Table of Contents

Program

Program

Monday, March 26, 2012

8:00-8:30 a.m. ***Registration and Poster Set-up***

8:30-8:45 a.m. ***Plenary Session I*** *(Main Auditorium)*

Opening Remarks and CCR-FYI Overview
Kristin Fabre, Ph.D., Chair, CCR-FYI Steering Committee

8:45-9:00 a.m. ***Opening Remarks from the CCR Office of Training and Education***
Jonathan Wiest, Ph.D., Associate Director, Office of Training and Education, Center for Cancer Research, NCI

9:00-9:30 a.m. ***CCR Office of the Director Address***
Robert Wiltrout, Ph.D., Director, Center for Cancer Research, NCI

9:30-10:30 a.m. ***Keynote Speaker I: "Design and Development of Therapeutic Cancer Vaccines"*** *(Main Auditorium)*
Jeffrey Schlom, Ph.D., NCI CCR Laboratory of Tumor Immunology and Biology, and Head of the Immunotherapeutics Group, NCI
Moderators: Alyson Freeman, Ph.D. and Balamurugan Kuppusamy, Ph.D.

10:45-a.m.
12:00 p.m. ***Concurrent Oral Presentations I***

I. Immunology I *(Room E1/E2)*

10:45 **Balamurugan Kuppusamy**, Laboratory of Cell and Developmental Signaling
FBXW7alpha attenuates inflammatory signaling by suppressing expression of Cebpd and its target gene Tlr4

11:00 **Mingqian Feng**, Laboratory of Molecular Biology
HN3: a human single-domain monoclonal antibody binds cell surface-associated glypican-3 and inhibits hepatocellular carcinoma cell proliferation

11:15 **Marco Cardone**, Laboratory of Experimental Immunology
Interleukin (IL)-1beta controls the Dectin-1mediated programming of human dendritic cells via IkappaB-zeta

11:30 **Jaba Gamrekelashvili**, Medical Oncology Branch
Tumor cells contain veto factors limiting immunogenicity of sterile necrosis and controlling CD8+ T cell activation

11:45 **Jinyao Li**, Vaccine Branch
Co-immunization with HIV env DNA and Protein Elicit Both Strong Cellular and Humoral Immune Responses

Moderators: Katie Stagliano, M.S. and Miranda Hanson, Ph.D.

II. Molecular and Cell Biology, Virology, and Bioinformatics I *(Balcony A)*

10:45 **Alexander Gibbons**, Laboratory of Cancer Biology and Genetics
Tempol as a potential protective agent of nucleoside reverse transcriptase inhibitor (NRTI)-induced mitochondrial toxicity

11:00 **Nicole Naiman**, HIV and AIDS Malignancy Branch
Oxidative stress upregulates the latency-associated nuclear antigen protein of Kaposis Sarcoma-Associated Herpesvirus in chronically infected cells

11:15 **Viraj Kulkarni**, Vaccine Branch
Altering the Immunodominance Hierarchy of p55gag by DNA Vaccine Expressing Conserved Regions

11:30 **Andrea Fischione**, Medical Oncology Branch
Cell cycle-dependent phosphorylation regulates BAG3 binding to Hsp70

11:45 **Rashmi Jalah**, Vaccine Branch
SIVmac239 DNA and Virus Particle Vaccination Confers Protection From Infection and Disease Progression Upon Challenge With Heterologous SIVsmE660

Moderators: Brajendra Tripathi, Ph.D. and Poorva Dharkar, Ph.D.

III. Chemistry, Pharmacology, and Structural Biology (Balcony B)

10:45 **Yuhong Chen**, Cancer and Inflammation Program
Fully synthetic virus-like nanoparticles targeting prostate cancer cells

11:00 **Emily Whitson**, Molecular Targets Laboratory
Synergistic TRAIL sensitizers from Barleria alluaudii and Diospyros maritima

11:15 **Suneet Shukla**, Laboratory of Cell Biology
3DQSAR studies of BCR-ABL kinase inhibitor, Tasigna (nilotinib) and its derivatives for identification of pharmacophore essential for interaction with ABC drug transporters and target kinases

11:30 **Mathieu Metifiot**, Laboratory of Molecular Pharmacology
Understanding HIV-1 Integrase and strand transfer inhibitors to overcome clinical resistance

11:45 **Yeong Sang Kim**, Chemical Biology Laboratory
Development of a high-throughput SUMOylation assay using fluorescent peptide probes and identification of SUMOylation inhibitors

Moderators: Adeola Makinde, Ph.D. and Fanching Lin, Ph.D.

IV. Genetics, Genomics, and Proteomics (Room F1/F2)

10:45 **Sichuan Xi**, Surgery Branch
miR487b is a Tumor Suppressor Silenced by Epigenetic Mechanisms during Tobacco-Induced Pulmonary Carcinogenesis

11:00 **McAnthony Tarway**, Laboratory of Translational Genomics
Risk alleles of four prostate cancer associated variants within the 8q24.2 region show increased interaction with androgen receptor

11:15 **Indu Kohaar**, Laboratory of Translational Genomics
Functional exploration of CCNE1 splicing forms and link to bladder cancer

11:30 **Nilabja Sikdar**, Laboratory of Translational Genomics
Identification and Functional Characterization of a Novel Splicing Form of the TPCN2 Gene on 11q13.3 Within a Region Associated with Prostate Cancer Risk

11:45 **Hui Yang**, Gene Regulation and Chromosome Biology Laboratory
Identifying and Cloning the Hard Stuff from MCF7 breast cancer Cell line: DNA Palindromes

Moderators: Ishminder Mann, Ph.D. and Jane (Xiao Hui) Tan, Ph.D.

V. Cancer Models, Metastasis, Microbiology, and Biophysics (Balcony C)

10:45 **Sarah Beachy**, Genetics Branch
Enforced Expression of Lin28b Results in Peripheral T Cell Lymphoma In Vivo

11:00 **Natascia Marino**, Laboratory of Molecular Pharmacology
Biochemical and functional interaction of two metastasis suppressors, Nm23-H1 and Gelsolin, in breast carcinoma cells

11:15 **Qun Jiang**, Laboratory of Experimental Immunology
IKKa links inflammation and tumorigenesis in lung squamous cell carcinoma

11:30 **Suhwan Chang**, Mouse Cancer Genetics Program
E3 ubiquitin ligase activity of human BRCA1 is necessary for its tumor suppression function

11:45 **Akiko Chiba**, Laboratory of Cell and Developmental Signaling
Functional Characterization of Rab31 Gene in Pancreatic Cancer Cell Tumorigenesis

Moderators: Maria Cristina Rangel, Ph.D. and Bethanie Morrison, Ph.D.

12:00-1:30 p.m. ***Poster Session I***

12:00–12:45 p.m. Odd-numbered Posters – presenter must be present
12:45–1:30 p.m. Even-numbered Posters – presenter must be present

- Immunology I
- Molecular and Cell Biology, Virology, and Bioinformatics I
- Chemistry, Pharmacology, and Structural Biology
- Genetics, Genomics, and Proteomics
- Cancer Models, Metastasis, Microbiology, and Biophysics

1:30-2:30 p.m. ***Special Highlight Presentation: "Cancer Survivorship" (****Main Auditorium)*
Joanna Rudnick, Owner/Producer at Joanna Rudnick Productions LLC
Moderators: Nesrin Rechache, Ph.D. and Maria Cristina Rangel, Ph.D.

2:30-3:30 p.m. ***Keynote Speaker II: "Functional Annotation of the Cancer Genome via RNAi"*** *(Main Auditorium)*
Natasha J. Caplan, Ph.D., Head of Gene Silencing Section, Genetics Branch, CCR, NCI
Moderators: John Simmons, B.S. and Alyson Freeman, Ph.D.

3:45-5:15 p.m. ***Concurrent Workshops I***

1. ***Preclinical Research and Development: From Discovery to Therapy*** *(Main Auditorium)*
Susan E. Old, Ph.D., Senior Advisor and Acting Scientific Deputy Director of the NIH Center for Translational Therapeutics (NCTT), NIH
Alice Chen, MD, FACP, Medical Officer, Investigational Drug Branch, CTEP, DCTD, NCI
Susan E. Bates, M.D., Senior Investigator, Medical Oncology Branch and Affiliates, Head, Molecular Therapeutics Section, NCI
Paul G. Kluetz, M.D., Medical Officer, Division of Drug Oncology Products (DDOP), OODP, OND, CDER, FDA
Moderators: John Simmons, B.S. and Anne Noonan, M.D.

2. ***Careers in Science Writing: Trading in the Pipette for the Pen*** *(F1/F2)*
Sean Sanders, Ph.D., Editor for Custom Publishing and Program Director for Outreach, Science/AAAS
Carol Torgan, Ph.D., Expert reviewer for HealthNewsReview.org and consultant
Susan Kralian, Ph.D., Founder Oncology Medical Writing Services, Inc.
Jennifer Crawford, Ph.D., Science writing contractor, NCI Office of Communications and Education
Allison Bierly, Ph.D., Technical Writer, Qiagen
Moderators: Jeffrey Zhao, Ph.D. and Nesrin Rechache, Ph.D.

3. ***Making Yourself Marketable*** *(E1/E2)*
Lori M. Conlan, Ph.D., Director of Postdoctoral Services, Office of Intramural Training and Education, NIH
Samantha Sutton, Ph.D., Vice President and Director of Courses and Seminar, Senior Coach, Handel Group
Randall Ribaudo, Ph.D., President, CEO, Human Workflows, LLC; Co-founder, SciPhD.com

Marc Kuchner, Ph.D., Research Astrophysicist, NASA/Goddard Space Flight Center
Moderators: Ravikiran Yedidi, Ph.D. and Janani Varadarajan, Ph.D.

5:15 p.m. ***ADJOURN***

5:45 p.m. ***Happy Hour*** *(Blackfinn Restaurant, Fairmont Ave., Bethesda, MD)*

Tuesday, March 27, 2012

8:00-8:30 a.m. ***Registration and Poster Set-up***

8:30-9:30 a.m. ***Keynote Speaker III: "Stem Cells in Lung Regeneration and Repair"***
(Main Auditorium)
Brigid Hogan, Ph.D., FRS, Professor and Chair, Department of Cell Biology, Duke University
Moderators: Kristin Fabre, Ph.D. and Katie Stagliano, Ph.D.

9:45-11:15 a.m. ***Concurrent Workshops II***

4. Careers Outside Academia – Industry, Business and Government *(Balcony A)*
Marguerite Camp Clarkson, Ph.D., Senior Associate, PricewaterhouseCoopers LLP, Washington Federal Practice
Rebecca Dunfee, Ph.D., Senior Consultant, Booz Allen Hamilton
Ramkumar Mandalam, Ph.D., President and CEO, Cellerant Therapeutics Inc.
Kurt Marek, Ph.D., Program Director , National Heart, Lung, and Blood Institute, NIH
Brock A. Peters, Ph.D., Associate Director of Research, Complete Genomics Inc.
Moderators: Barbara Rath, Ph.D. and Ishminder Mann, Ph.D.

5. Careers in Academia and Grants Management *(E1/E2)*
Nancy C. Lohrey, M.S., M.T.-ASCP, Program Director, Grant Awards, Cancer Training Branch, NCI-CCT/NIH
Margarita (Magui) Cardona, M.S., C.R.A., Director of Sponsored Research, University of Baltimore, MD
Ram Savan, Ph.D., Assistant Professor, Department of Immunology, University of Washington, Seattle, WA
Ancha Baranova, Ph.D., Associate Professor, Molecular and Microbiology Department, George Mason University, Manassas, VA
Craig Laufer, Ph.D., Professor of Biology, Hodson Science & Technology Center, Hood College, Frederick, MD
Moderators: Poorva Dharkar, Ph.D. and Jacqueline Salotti, Ph.D.

6. Communicating Science: Getting the Message Out *(Balcony C)*
Evonne Kaplan-Liss MD, MPH, FAAP, Director, Advanced Certificate in Health Communications, Research Assistant Professor of Preventive Medicine, Graduate Program in Public Health, School of Journalism, State University of New York at Stony Brook
Moderators: Maria Cristina Rangel, Ph.D. and Janani Varadarajan, Ph.D.

11:30 am-
12:45 p.m. ***Concurrent Oral Presentations II***

I. Immunology II *(Room E1/E2)*

11:30 **Andrew Stewart**, Cancer Inflammation Program
IL-10 From Tumor T Regulatory Cells Is Induced By Type I Interferon And Limits The Local Th-17 Response

11:45 **Dror Luger**, Laboratory of Cancer Biology and Genetics
Tumor-Induced CD79 Expressing Myeloid Cells Are Associated With The Metastatic Process In Mouse Models Of Breast Cancer

12:00 **Anna Nam**, Experimental Immunology Branch
Transgenic interleukin-6 enhances peripheral T cell survival and homeostasis

12:15 **Nirali Shah**, Pediatric Oncology Branch
A Pilot Trial of Wilms tumor 1 (WT1) Peptide-Loaded Allogeneic Dendritic Cell (DC) Vaccination and Donor Lymphocyte Infusions (DLI) for WT1-Expressing Hematologic Malignancies Relapsing after Allogeneic Hematopoietic Stem Cell Transplantation (alloHSCT)

12:30 **Paul Su**, Pediatric Oncology Branch
Immunosuppression of the Host Immune System in Acute Lymphoblastic Leukemia: an insight into the role of PD-1

Moderators: Muthukumar Balasubramaniam, Ph.D. and Sagar Kudchodkar, Ph.D.

II. Molecular and Cell Biology, Virology, and Bioinformatics II (Balcony B)

11:30 **Marco Mineo**, Medical Oncology Branch
Chronic myeloid leukemia (CML) exosomes promote angiogenesis in a Src-dependent fashion in vitro and in vivo

11:45 **Philip Tedbury**, HIV Drug Resistance Program
ADP-Ribosylation Factor 1 in HIV-1 Assembly and Release

12:00 **Bing Yu**, Molecular Oncology Branch
Protein SUMOylation is synthetic lethal to oncogenic KRAS in colorectal cancer cells

12:15 **Ina O'Carroll**, HIV Drug Resistance Program
Functional Redundancy in HIV-1 Assembly

12:30 **Vijay Walia**, Laboratory of Cell and Developmental Signaling
Lysophosphatidic acid signaling regulates primary cilia assembly

Moderators: Balamurugan Kuppusamy, Ph.D. and Ravindra Veeranna, Ph.D.

III. Translational, Clinical, and Epidemiology Research (Balcony A)

11:30 **Daniel Lee**, Pediatric Oncology Branch
Surprising Eradication of Pediatric ALL by CD4+ T cells with a CD19-Specific Chimeric Antigen Receptor

11:45 **Holger Pflicke**, Surgery Branch
Dual PI3K/mTOR inhibition induces cell death in a defined subset of pancreatic cancer cell lines

12:00 **Prashant Tembhare**, Laboratory of Pathology
CD81: A Novel, Specific and Highly Sensitive Marker in Flow Cytometric Diagnosis of Plasma Cell Dyscrasia

12:15 **David Soto Pantoja**, Laboratory of Pathology
Deficiency of CD47 in the tumor microenvironment enhances tumor responses to ionizing radiation, preserves immunosurveillance, and protects normal tissue through an increase in autophagy

12:30 **Yi-Ping Fu**, Laboratory of Translational Genomics
Common genetic variants in the PSCA gene influence gene expression and bladder cancer risk

Moderators: Nesrin Rechache, Ph.D. and John Simmons, B.S.

IV. Signal Transduction, Transcription, and Chromatin (Room F1/F2)

11:30 **Stephanie Morris**, Laboratory of Receptor Biology and Gene Expression
Multiple Chromatin Remodeling Systems Contribute to Dynamic Transitions in Chromatin Structure

11:45 **Elisabetta Leo**, Laboratory of Molecular Pharmacology
Analysis of DNA Replication in Cancer Cells upon Knockdown of Licensing and Initiation Factors

12:00 **Su-Ryun Kim**, Laboratory of Cell and Developmental Signaling
C/EBPδ promotes nuclear localization of p21CIP1 and cytotoxicity of tamoxifen in ER(+) breast tumor cells

12:15 **Aparna Kotekar**, Experimental Immunology Branch
Bidirectional Transcription of the Upstream Regulatory Region may serve a Regulatory Role in MHC Class I Transcription

12:30 **Ya Zhang**, Laboratory of Molecular Pharmacology
A mammalian replicator binding protein essential for sequence-specific initiation of DNA replication

Moderators: Jeffrey Zhao, Ph.D. and Jacqueline Salotti, Ph.D.

V. Cancer Prevention, Carcinogenesis, and Cancer Stem Cells (Balcony C)

11:30 **Wei Liu**, Basic Research Laboratory
The reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by c-MYC

11:45 **Kian-Huat Lim**, Medical Oncology Branch
Oncogenic TLR-MyD88 Signaling In Activated B-Cell-Like Diffuse Large B-Cell Lymphoma: Mechanism and Potential Therapeutic Strategies

12:00 **Zhewei Tang**, Laboratory of Molecular Biology
A single-domain human antibody elicits potent antitumor activity by targeting an epitope in mesothelin close to the cancer cell surface

12:15 **Lei Sun**, Laboratory of Cancer Prevention
Epigenetic Regulation of CpG Promoters by the NF-κB Signaling Pathway in Pancreatic Cancer Stem Cells

12:30 **Cody Schlaff**, Radiation Oncology Branch
Epigenetic modulation of glioma stem-like cells promotes decreased expression of CD133 and induces differentiation

Moderators: Barbara Rath, Ph.D. and Bethanie Morrison, Ph.D.

12:00-5:00 p.m. ***Career Fair***

12:45-2:15 p.m. ***Poster Session II***

12:45–1:30 p.m. *Odd-numbered Posters – presenter must be present*
1:30–2:15 p.m. *Even-numbered Posters – presenter must be present*

- Immunology II
- Molecular and Cell Biology, Virology, and Bioinformatics II
- Translational, Clinical, and Epidemiology Research
- Signal Transduction, Transcription, and Chromatin
- Cancer Prevention, Carcinogenesis, and Cancer Stem Cells
- Core Services

7. Navigating Work Relationships: Recognizing Conflict Pitfalls and Opportunities *(Main Auditorium)*

2:15-3:45 p.m. **Howard Gadlin, Ph.D.**, NIH Office of the Ombudsman, Center for Cooperative Resolution, NIH
Linda Myers, J.D., Associate Ombudsman, NIH Office of the Ombudsman, NIH

Moderators: Kristin Fabre, Ph.D. and Majda Haznadar, Ph.D.

3:45-4:45 p.m. ***Postdoctoral Keynote Speaker: "Tumor Heterogeneity and Personalized Therapy in Liver Cancer"*** *(Main Auditorium)*

Junfang Ji, M.D., Ph.D., Research Fellow, Laboratory of Human Carcinogenesis, NCI

Moderators: Brid Ryan, Ph.D. and Jeffrey Zhao, Ph.D.

4:45-5:00 p.m. ***TRAVEL AWARDS*** *(Main Auditorium)*

5:00-5:10 p.m. ***Closing Address*** *(Main Auditorium)*
Francis S. Collins, M.D., Ph.D., Director, NIH

5:15 p.m. ***ADJOURN***

5:45 p.m. ***Happy Hour*** *(Blackfinn Restaurant, Fairmont Ave., Bethesda, MD)*

Introductory and Keynote Presentation Speakers

Kristin Fabre, Ph.D.
Chair, CCR-FYI Steering Committee

Dr. Kristin Fabre is currently a Postdoctoral Fellow in the Radiation Biology Branch of the Center for Cancer Research, National Cancer Institute. Dr. Fabre received her BS in Biology from the University of Wyoming, followed by an MS and PhD from Colorado State University in Cell and Molecular Biology. While at CSU, she studied radiation biology, specifically the impact of DNA repair polymorphisms on radiation-induced breast cancer.

She came to the NCI in 2008 and joined Dr. James B. Mitchell whose laboratory studies compounds that potentially produce biological responses to radiation. Her areas of interest include looking at natural-based compounds, such as resveratrol and fish oil, as antioxidants and radioprotectors. Furthermore, she has projects pertaining to probiotics and gut microflora in response radiation and nitroxides. In collaboration with pharmaceutical companies, she also studies the effects of newly developed chemotherapeutic drugs to determine their efficacy as radiosensitizers.

Jonathan Wiest, Ph.D.
Director, Center for Cancer Training,
Center for Cancer Research, NCI

Dr. Jonathan S. Wiest obtained a bachelor's degree in analytical chemistry from the University of Wisconsin-Milwaukee in 1980. He worked as a production chemist synthesizing oligonucleotides for P-L Biochemicals until he began graduate school in 1982 at the Medical College of Ohio in Toledo. Dr. Wiest received a Ph.D. in Biochemistry in 1988 and then did a Postdoctoral fellowship at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina. He rose to the rank of Senior Staff Fellow and then assisted in establishing a Cancer Research Institute in Western Colorado. In 1996 he became an assistant professor at the University of Cincinnati, Department of Environmental Health, School of Medicine. Dr. Wiest joined the Center for Cancer Research at the National Cancer Institute (NCI) as the Associate Director for Training and Education in November of 2001. In 2007 Dr. Wiest was appointed by the NCI Director to serve as the Acting Director for the Cancer Prevention Fellowship Program and in early 2008 the NCI Director also appointed Dr. Wiest to lead the formation of the Center for Cancer Training (CCT) as the Dierctor. The CCT is charged with coordinating the major training activities in the NCI in both the Intramural and Extramural communities. In 2003, Dr. Wiest received the NIH Driector's award for mentoring as well as the NCI Outstanding Mentor Award. In November 2007 he received an NIH award of Merit for mentoring. The major focus of his research involves generic alterations in lung tumorigenesis. He is involved in studies to identify tumor suppressor genes and altered signaling pathways in lung cancer.

Robert H. Wiltrout, Ph.D.
Director, Center for Cancer Research, NCI

Dr. Robert H. Wiltrout is the Director of the Center for Cancer Research (CCR), home to the basic, clinical, and translational research enterprise located within the National Cancer Institute in Bethesda and Frederick, Maryland. He serves as a member of NCI's Scientific Program Leaders Committee, co-chairs the Scientific Advisory Committee for NCI's Experimental Therapeutics Program, and chairs CCR's Center of Excellence in Immunology.

Dr. Wiltrout has authored more than 200 papers in the areas of innate immune response regulation, cytokine biology, and the biological therapy of renal cell carcinoma. His early research studied mechanisms of in vivo regulation of natural killer (NK) cells and the role of NK cells in limiting the formation and progression of metastases in major non-lymphoid organs such as the lungs and liver. His laboratory has also elucidated novel effects and interactions of NK and NKT cells on inflammation and tumor outcome in the liver. The team has contributed new insights into the in vivo functions of the CD40 co-stimulatory molecule expressed on both antigen presenting cells and on renal cancer cells. Recently they have shown that combinations of IL-2 with agonist CD40 antibody can have profound IL-12-dpendent antitumor effects, while also influencing the quality of both primary and secondary immune responses against metastatic kidney cancer. Dr. Wiltrout's laboratory has also translated basic science findings in the area of Cytokine biology into several completed or ongoing clinical trials to assess the effects of IL-2 and IL-12; they are currently contributing to NCI's early phase development of IL-15.

Dr. Wiltrout received a B.A. degree from Kutztown University, Kutztown, PA in 1972, a M.S. in Microbiology from the Pennsylvania State University, University Park, PA in 1975, and a Ph.D. in Immunology from Wayne State University School of Medicine, Detroit, MI in 1979.

Dr. Wiltrout has been the recipient of two NIH merit awards, a Career Achievement Award from the Department of Health and Human Services, and an award as Federal Laboratory Director of the year by the Federal Laboratory Consortium for the mid-Atlantic region in 2009, and nationally in 2010.

Jeffrey Schlom, Ph.D.
Chief, Laboratory of Tumor Immunology and Biology, Center for Cancer Research, NCI

Dr. Schlom conducts translational research in the area of cancer immunotherapy, with an emphasis on the design and development of recombinant vaccines for the therapy of human carcinomas. He emphasizes the use of these vaccines not only as a monotherapy but also in combination with other therapeutic modalities. Emphasis is placed on the seamless transition of hypothesis-based preclinical studies to science-based clinical trials. Dr. Schlom is Chief of the Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, NIH. He serves on the editorial boards of numerous scientific journals. He has authored more than 650 scientific publications and holds numerous patents for monoclonal antibody and recombinant vaccine generation and uses.

Natasha J. Caplen, Ph.D.
Head/Senior Scientist, Gene Silencing Section, Genetics Branch, Center for Cancer Research, NCI

Dr. Caplen obtained her Ph.D. from the University of London (Kings College Hospital Medical School) for studies on the genetics of type I diabetes and its complications. Dr. Caplen's Postdoctoral training began at St Mary's Hospital Medical School, Imperial College, London, where she focused on the development of gene therapy approaches for cystic fibrosis and was involved in some of the first pre-clinical and clinical studies of cationic lipid mediated gene therapy for cystic fibrosis. In 1996, Dr. Caplen came as a Visiting Fellow to the National Human Genome Research Institute (NHGRI) at NIH where she initially conducted studies investigating hybrid viral vector systems for the delivery of genes. It was while at NHGRI that Dr. Caplen developed a research interest in the newly identified gene silencing mechanism RNA interference (RNAi), leading to her studies that help establish the presence of RNAi in mammalian cells. Dr. Caplen joined CCR, NCI in 2004 as a Senior Scientist and leads the Gene Silencing Section in the Genetics Branch.

Dr. Caplen's research at the NCI is focused on using the molecular and phenotypic perturbations induced by RNAi to gain insight into the biology of cancer, to identify novel anti-cancer molecular targets, and to discover and functionally validate genes that modulate the activity of cancer therapeutics. She is also investigating the role specific non-coding RNAs/miRNAs play in cancer. Dr. Caplen has published over 60 peer-reviewed papers, reviews and book chapters, initially within the gene therapy research field and, more recently, on the role and exploitation of the RNAi mechanism in mammalian cells. These include first author articles in Nature Medicine, and P.N.A.S.; both of these studies have been cited in the literature >500 times each. Most recently she has published studies using RNAi screens to discover genes related to the biology of colorectal cancer and breast cancer and the identification of genes that enhance the activity of cancer drugs associated with the induction of DNA damage. Dr. Caplen's group has also published several studies investigating the role miRNAs play in the regulation of cancer-associated genes, including a recent study establishing the receptor tyrosine kinase AXL as a functionally relevant target of miR-34a in breast cancer. Dr. Caplen received a NCI Director's Mentor of Merit Award in 2009 and a NIH Director's Award in 2011 for her role in establishing the Intramural Trans-NIH large-scale RNAi screening facility.

Brigid L.M. Hogan, Ph.D., FRS
Professor and Chair, Department of Cell Biology, Duke University

Brigid Hogan, PhD, FRS is the George Barth Geller Professor and Chair of the Department of Cell Biology, Duke University Medical Center. She is also Director of the Duke Stem Cell Program. Prior to joining Duke, Dr. Hogan was an Investigator of the Howard Hughes Medical Institute and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University Medical Center. Dr. Hogan earned her PhD in Biochemistry at the University of Cambridge. After completing her PhD, she was a postdoctoral fellow in the Department of Biology at MIT. Before moving to the United States in 1988 Dr Hogan was head of the Molecular Embryology Laboratory at the National Institute for Medical Research in London. Her research currently focuses on the genetic control of embryonic development and morphogenesis, using the mouse as a model system. She currently has a particular interest in stem cells of adult endodermal organs, including the lung and esophagus, and their role in organ turnover and repair. She was President of the American Society for Developmental Biology and is President of the American Society of Cell Biology. Her service to the scientific community has included being a member of the National Advisory Council of the National Institute of Child Health and Human Development, Co-Chair for Science of the 1994 NIH Human Embryo Research Panel and a member of the 2001/2002 National Academies Panel on Scientific and Medical Aspects of Human Cloning. Dr. Hogan is a Fellow of the Royal Society of London and the American Academy of Arts and Sciences, Past-President, American Society of Cell Biology, and a member of the Institute of Medicine and the National Academy of Sciences, USA.

Junfang Ji, M.D., Ph.D.
Research Fellow, Laboratory of Human Carcinogenesis, Center for Cancer Research, NCI

Dr. Junfang Ji grew up in China. She received her Bachelor degree in Medicine from Shanxi Medical University in 2000 and Master degree in Medicine from Medical College of Zhengzhou University in 2003. In 2006, she got her Ph.D. degree in Molecular Oncology from Chinese Academy of Medical Science & Peking Union Medical College. During her master and doctor degrees period, her studies focused on molecular mechanisms on tumor initiation and her papers had been published in Molecular Cellular Biology, Oncogene, etc. After that, she joined Dr. Xin Wei Wang' group at NCI/NIH for her post-doctoral fellowship in 2006. She was promoted to Research Fellow in 2011. Her studies in NIH focus on tumor heterogeneity and personalized therapy in liver cancer. Till today, her work has led to 12 publications in high impact journals including New England Journal of Medicine and Gastroenterology, 3 book chapters and 2 patents owned by U.S. Government.

Francis S. Collins, M.D., Ph.D., Director, NIH

Francis S. Collins, M.D., Ph.D. is the Director of the National Institutes of Health (NIH). In that role he oversees the work of the largest supporter of biomedical research in the world, spanning the spectrum from basic to clinical research.

Dr. Collins is a physician-geneticist noted for his landmark discoveries of disease genes and his leadership of the international Human Genome Project, which culminated in April 2003 with the completion of a finished sequence of the human DNA instruction book. He served as director of the National Human Genome Research Institute at the NIH from 1993-2008.

Before coming to the NIH, Dr. Collins was a Howard Hughes Medical Institute investigator at the University of Michigan. He is an elected member of the Institute of Medicine and the National Academy of Sciences, was awarded the Presidential Medal of Freedom in November 2007, and received the National Medal of Science in 2009.

Workshop Descriptions and Presenters

1. Preclinical Research and Development: From Discovery to Therapy

The aim of this workshop is to provide a comprehensive overview of how the discoveries in the field of basic science are translated into real-world therapeutic agents that can benefit patients with cancer. The workshop will highlight the key steps involved in choosing the appropriate target, designing compounds and performing pre-clinical toxicology assessments, appropriate design of clinical trials, assessing the results of clinical trials, and issues involved in the drug approval process. Opportunities for careers at the various stages will be emphasized.

Susan E. Old, Ph.D.

Dr Susan Old is Senior Advisor and Acting Scientific Deputy Director of the NIH Center for Translational Therapeutics (NCTT) at the National Institutes of Health. The NCTT, which includes the NIH Chemical Genomics Center (NCGC), the Therapeutics for Rare and Neglected Diseases (TRND) program, and systems-wide initiatives in toxicology and RNAi, comprises a comprehensive program of innovation to translate the Human Genome Project into therapeutic benefits for human health. The NCTT's collaborative research projects bridge the substantial gap in time and resources that exists between basic research and human testing of new drugs.

Dr. Old received her undergraduate degree at the University of California, Irvine, and her Ph.D. in the Department of Human Genetics at the University of Michigan. She completed her post-doctoral training at Columbia University in Pediatric Neurology before joining the NIH in 1988. She began her career at NIH as a senior staff fellow at the National Eye Institute, where she studied the mechanisms of diabetic cataracts in the Laboratory of Mechanisms of Ocular Disease. In 1994, she joined the extramural program at the National Heart, Lung, and Blood Institute (NHLBI), where she was responsible for establishing programs in genomics and proteomics. In 2006 she became the Acting Deputy Director for the Division of Cardiovascular Diseases and was responsible for an extramural budget of more than $1.3 billion. Prior to joining the NIH Center for Translational Therapeutics, she was the Senior Advisor to the Director for Translational Research at the National Center for Research Resources (NCRR).

Alice Chen, MD, F.A.C.P.

Dr. Chen is currently a Medical Officer in the Target Therapeutics 1 Section of the Investigational Drug Branch of the Cancer Therapy Evaluation Program of the National Cancer Institute. She is board certified in Internal Medicine and Medical Oncology. She has served as a primary and associate investigator in a broad spectrum of clinical trials ranging from phase 0 through phase 4. Her portfolio includes DNA binding agents and angiogenesis, integrin, HSP 90, PARP and topoisomerase inhibitors. Dr. Chen is also the CTEP contact for revision 4 of the Common Terminology Criteria for Adverse Events.

Susan E. Bates, M.D.

Dr. Susan Bates received her M.D. from the University of Arkansas School of Medicine. She completed her clinical training in internal medicine at Georgetown University in Washington, D.C., and in oncology at the NCI. Dr. Bates has held positions of increasing responsibility at the NCI, receiving tenure in 1992. Her interests range from clinical studies on drug resistance to laboratory studies on drug resistance in breast cancer and renal cell cancer. Her Molecular Therapeutics Section is dedicated to finding antineoplastic agents that, alone or in combination with other anticancer agents, improve cancer therapy. Her section is focused on new drug development. Dr Bates notably developed depsipeptide FR901228, a novel histone deacetylase inhibitor which was recently approved by the FDA for the treatment of cutaneous and peripheral T-cell lymphomas.

Paul G. Kluetz, M.D.

Dr. Paul Kluetz is a graduate of the University of Maryland Medical School. He did his internal medicine training at the University of Maryland Medical Center. He completed his medical oncology fellowship at the National Cancer Institute, where he conducted phase 1 and II clinical trials in prostate cancer. Dr. Kleutz is currently a Medical Reviewer at the FDA. He is involved in the review of new oncology drug applications for marketing approval in the United States. His specific disease interest is prostate cancer.

2. Careers in Science Writing: Trading in the Pipette for the Pen

Do you enjoy writing about science and reviewing manuscripts? Are you searching for a career which combines scientific knowledge with your more creative side? Do you want to make science easier to understand for the general public? Then maybe a career in science writing is for you.

This workshop will provide participants information regarding the variety of science writing career paths. You will learn from the panelists that both formal and on-the-job training can lead to exciting careers in science writing. All panelists attending the workshop started out as PhD bench scientists but later traded in the pipette for the pen. Their experiences range from editing for Science, developing web and mobile-based interactive educational programs, freelance science writing, to writing for the government, pharmaceutical, and biotechnology companies. They will share with you their experiences in the science writing field and provide insights on how to successfully make the transition into a science writing career.

Sean Sanders, Ph.D.

Dr. Sanders did his undergraduate training at the University of Cape Town, South Africa, and his Ph.D. at the University of Cambridge, UK, supported by the Wellcome Trust. Following postdoctoral training at the National Institutes of Health and Georgetown University, Dr. Sanders joined TranXenoGen, a startup biotechnology company in Massachusetts working on avian transgenics. Pursuing his parallel passion for writing and editing, Dr. Sanders joined BioTechniques as an editor, before joining Science/AAAS in 2006. Currently Dr. Sanders is the Editor for Custom Publishing for the journal Science and Program Director for Outreach.

Carol Torgan, Ph.D.

Dr. Carol Torgan received her Ph.D. in Kinesiology from the University of Texas, was an assistant research professor at Duke University Medical Center, and then served as a research fellow in the NHLBI intramural Laboratories of Biochemical Genetics and Cell Biology. While at NIH, Carol traded in the lab bench for a lap top in order to translate scientific information to a broader audience. She then joined the start-up Revolution Health as senior content director, where she designed strategies to blend evidence-based information and Web 2.0 tactics into actionable content. Carol has received funding for her research from NIH and NASA, and has received plain language writing awards for her communications outreach. She currently serves as an expert reviewer for HealthNewsReview.org, and consults for a number of agencies, including the NIH. Her projects include developing Web- and mobile-based interactive educational programs, crafting e-newsletters for health professionals and patients, and advising on digital content strategies and mobile health trends.

Susan Kralian, Ph.D.

Susan Kralian's PhD is in the area of neuroscience and behavior from the University of Massachusetts, Amherst. She also has a BA in biology from the University of Colorado, Boulder. Immediately after obtaining her PhD, Susan Kralian began a position as a sabbatical replacement visiting Assistant Professor in biology at Rocky Mountain College in Montana. The following year, she taught at the University of Idaho, Pocatello, as a lecturer in the biological sciences department. She taught a wide variety of courses at these institutions, such as introduction to biology to graduate level clinical physiology courses. Susan Kralian then left academia and moved to New York City, New York. Here, she began her career as a medical writer and worked at various medical communication companies. Later, in 2009, she founded Oncology Medical Writing Services, Inc., and has worked for the last 3 years as an independent medical communication specialist/freelance medical writer. Susan Kralian primarily develops and writes material in the area of oncology or neuroscience.

Jennifer Crawford, Ph.D.

Jennifer Crawford received a bachelor's degree in chemistry from Rose-Hulman Institute of Technology and a doctorate in biochemistry from the University of Kentucky. She spent two years as a post-doctoral fellow in the NCI's Mammary Biology and Tumorigenesis Laboratory and then joined the NCI's Health Communications Internship Program. She spent six months in the Center for Cancer Research Office of Communications and six months in the NCI Office of Communications and Education writing about recently published biomedical research. She is currently employed by Kelly Services as a science writing contractor in the NCI Office of Communications and Education.

Allison Bierly, Ph.D.

Allison Bierly is a technical writer at QIAGEN in Frederick, Maryland. She started out as a Biology and English double major at Ursinus College and received her PhD from Cornell University, studying immunity to Toxoplasma gondii, a protozoan pathogen. She came to NCI-Frederick in 2010 and stayed for a year and a half before moving into her first writing position, and has been at QIAGEN for almost 7 months now.

3. Making Yourself Marketable

In these tough economic times when the job market is highly competitive, setting yourself apart from the crowd is essential to securing your dream job. Doing good science and publishing will get you only so far – you will have to market and sell yourself in ways that are attractive to the employer. Developing and practicing that skill is more of an art than a science.

This skill-building workshop will help you chart out the essential steps to take for successfully moving ahead in the career path of your choice. Specifically, you will learn about the tools you need to map out your career path, develop your professional profile, and position yourself for success. The panelists will provide guidance and tips on wide range of topics including setting goals and achieving them, networking efficiently, interviewing effectively, and giving successful job talks.

Lori M. Conlan, Ph.D.

Dr. Lori M. Conlan is trained as a biochemist, receiving her B.S. in biochemistry from Michigan State University and her Ph.D. in biochemistry and biophysics from Texas A&M University. She worked for several years as a postdoc at the Wadsworth Center, NYS Department of Health, before transitioning from the lab to focus on career issues for the next generation of scientists. Dr. Conlan started as the director of the Science Alliance, an international career development program for graduate students and postdocs sponsored by the New York Academy of Sciences. She now is at the NIH in the Office of Intramural Training & Education assisting the 4000 NIH postdocs in their personal career choices. She organized many workshops at NIH helping the young trainees to improve their awareness about career goals, career planning, etc. She also organized career fairs for the intramural community that helped many trainees to succeed in their future goals. She speaks at universities and institutions around the nation on career development topics for young scientists. Additionally, she volunteers as a Board member for the National Postdoctoral Association (NPA).

Samantha Sutton, Ph.D.

Dr. Samantha Sutton's mission is to accelerate and globalize the next stage of human development: conscious life-design, one person at a time. She teaches that people are capable of living lives that make them happy and proud, so long as they have the tools to overcome limiting thoughts, excuses, beliefs and character traits and design new ones that work better. Dr. Sutton earned a Ph.D. in Biological Engineering at MIT as a Howard Hughes Pre-Doctoral Fellow, and a BS in Electrical Engineering at the University of Illinois at Urbana-Champaign. She then decided to aim her creativity, sense of scientific inquiry, love of humanity and structured engineering outlook to help people engineer better lives for themselves. As Vice President of Courses and Seminars, she has built the Life Coaching Crash Course into the Handel Group's® flagship course offering, which is now a large-scale course that is held in six cities and annually impacts hundreds of participants. While scaling up such programs, she has mindfully preserved a personalized, supportive experience for clients that is at the heart of HGLC coaching. The scope of her impact reaches into the academic institutions (Stanford University, MIT, Scripps Research Institute) and corporations such as BASF, DaVita Inc. and the US Government, to name a few. She has been invited as a guest speaker at

multiple conferences. She couples the analytical and problem-solving skills of a scientist and engineer with the care and insight of a coach and advisor to create a truly unique and impactful coaching style. Dr. Sutton has complemented her HGLC training with yoga, studies at a Buddhist monastery and mediation training. From her international volunteer experiences, she learned how to tailor messages to suit people of different cultural backgrounds. As a result, one of her career goals is to bring the Handel Method® to other countries in culturally appropriate ways.

Randall Ribaudo, Ph.D.

Human Workflows and SciPhD co-founder, Dr. Randall Ribaudo has over twenty years of experience in the Scientific Research and biotechnology field and has successfully made the transition from academia to industry. After receiving a Ph.D. in Immunology at the University of Connecticut, Dr. Ribaudo joined the Laboratory of Immunology, NIAID at NIH where he studied the molecular basis of antigen presentation. Dr. Ribaudo then accepted a position in the National Cancer Institute in the Laboratory of Immune Cell Biology as a Principal Investigator where he developed his own research program studying the immune response to viruses and tumors, as a team leader. His work at the NCI led to the development of a novel technology to develop vaccines against tumors and viruses for which, he holds patents that are now being further developed by private companies. During his time at Celera, Dr. Ribaudo has acted as a liaison between Celera and the pharmaceutical, biotechnology and academic communities, served as product manager responsible for developing support products for the Proteomics Groups mass spectrometry software, led the iScience Task Force to define strategic directions for sister company Applied Biosystems, advised on product development for the Celera Discovery System and enterprise solutions for information integration, and worked as a Manager of Strategic Solutions in the Informatics business. Prior to Celera, Dr. Ribaudo worked at the biotechnology and bioinformatics company Molecular Applications Group where he was responsible for presenting the revolutionary capabilities of MAG's products to representatives in the pharmaceutical, biotechnology and academic communities. All of this experience has provided Dr. Ribaudo with tremendous insight into the rapidly exploding technological capabilities in areas of discovery research, information and data management, as well as a detailed understanding of the human workflows that are required to successfully implement those technological solutions.

Marc Kuchner, Ph.D.

Dr. Marc Kuchner is an astrophysicist at NASA's Goddard Space Flight Center and a country songwriter. He is the co-inventor of the band-limited coronagraph, a tool for finding planets around other stars that will be part of the James Webb Space Telescope. He is also known for his work on planets with exotic chemistries: ocean planets, helium planets, and carbon planets. Dr. Kuchner took a year off from graduate school to intern in a Los Angeles recording studio that turned out to be a hotbed of country music. Since then, Dr. Kuchner has devoted much of his spare time to writing country songs in Nashville, TN. During these adventures in the music business, he developed an interest in marketing and realized that he could apply what he was learning in Nashville to help scientists. He enlisted the help of a few hundred of his colleagues on a Facebook group to try to piece together a new picture of how good marketing and good science can go hand in hand. The result is Marketing for Scientists: How to Shine in Tough Times, published by Island Press in November 2011. Dr. Kuchner received his bachelor's degree in physics from Harvard and his Ph.D. in astronomy from Caltech. He was awarded the 2009 SPIE early career achievement award for his work on coronagraphy. He has contributed to more than 100 research papers and published articles in journals including the Astrophysical Journal, Nature, and Astrobiology. He appears as an expert commentator in the Emmy nominated National Geographic television show "Alien Earths" and frequently writes articles in Astronomy Magazine.

4. Careers outside academia– Industry, Business and Government

The cuts in funding for academia and the more and more difficult process of an academic career path has led many life science PhDs to look for career opportunities outside academia. Skills acquired while conducting bench work are transferrable to many rewarding careers in business, industry and government organizations. To shed light on the various employment opportunities, career path and how to make successful transition, the workshop "Careers outside the lab" at the 12th CCR/FYI Colloquium 2012 includes a panel of professionals from various consulting/biotechnology companies and government organization. The panelists will discuss opportunities available in business, industry and government and skills required for successful transition to different career paths.

Marguerite Camp Clarkson, Ph.D.

Dr. Clarkson is a Senior Associate in the PricewaterhouseCoopers LLP (PwC) Washington Federal Practice. Prior to joining PwC, Dr. Clarkson was a post-doctoral fellow at the National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism (NIAAA) for over three years. At NIH she was the project leader on multiple experiments designed to investigate the underlying neuronal mechanisms of alcoholism and post traumatic stress disorder, and collaborated with colleagues, both nationally and internationally, to complete thorough, cutting-edge experiments. At PwC Dr. Clarkson's primary client is NIH. She works with ICs on various projects ranging from complex budget assessments on long-term health studies, to identifying, planning and implementing initiatives focused on organizational improvement and maturity. Dr. Clarkson received her Ph.D. in Behavioral Neuroscience from The University of Texas at Austin and her BA in Psychology from The University of North Carolina at Chapel Hill.

.

Ramkumar Mandalam, Ph.D.

Dr. Ramkumar Mandalam is the President and CEO of Cellerant Therapeutics Inc, a clinical stage biotechnology company developing novel cell-based and antibody therapies for cancer treatment and blood-related disorders. Prior to joining Cellerant in 2005, he was the Executive Director of Product Development at Geron Corporation, a biopharmaceutical company in Menlo Park, CA. His responsibility at Geron included strategic planning and implementation of development and manufacturing of cellular products for regenerative medicine, oncology and drug discovery applications. Dr. Mandalam served as the Director of Developmental Research at Aastrom Biosciences managing research and development programs involving ex vivo expansion of human primary cells for cell therapy applications. Dr. Mandalam received his Ph.D. in Chemical Engineering from the University of Michigan, Ann Arbor, Michigan.

Kurt Marek, Ph.D.

Dr. Marek is a Program Director at the National Heart, Lung, and Blood Institute (NHLBI) where he coordinates the Small Business Innovation Research (SBIR) program. In this role, he develops, manages, and evaluates scientific programs to support small businesses performing research and development on innovative biomedical products. Dr. Marek provides advice to small businesses on all aspects of the SBIR program, including funding opportunities, the application process, and commercializing technologies. In addition, he serves as an expert resource to NHLBI staff on the policies and regulations governing the SBIR program. Dr. Marek began working at the NHLBI in 2010 as an AAAS Science and Technology Policy Fellow.

Prior to beginning his fellowship at the NIH, Dr. Marek was a Damon Runyon Cancer Research Fellow at the University of California, San Diego where he studied the development of the spinal cord, using genomics to identify new roles for electrical activity in the nervous system and characterizing the underlying molecular mechanisms. Dr. Marek received his PhD in Neuroscience from the University of California, San Francisco where he was a Howard Hughes Predoctoral Fellow. For his thesis research, he used the fruit fly Drosophila to study synaptic development and function. He has a BS in Biology and a BA in Humanities from the University of California, San Diego.

Brock A. Peters, Ph.D.

Dr. Peters is Associate Director of Research at Complete Genomics Inc., a biotechnology company dedicated to complete human genome sequencing and analysis. Dr. Peters received his Ph.D. in Pharmacology from Johns Hopkins University School of Medicine, Baltimore, MD in 2005. After postdoctoral training at

Johns Hopkins University for less than a year, Dr. Peters joined Genentech as an Associate Scientist with the Department of Molecular Biology, where he was head of the sequencing effort for Genentech's Cancer Genome Project (CGP) and discovered a number of previously unreported genetic changes in cancer genomes. In 2008 he joined the Research Department at Complete Genomics Inc. as a Senior Scientist where he invented a novel method for fragmenting DNA prior to sequencing library preparation and led the team that developed a method for sequencing and haplotyping genomic DNA called Long Fragment Read (LFR) technology. After three years of being a team leader in the Research Department of Complete Genomics Inc., he moved on to his current position within the company as Associate Director of Research. In his current position, Dr. Peters manages over 10 collaborative projects between Complete Genomics and academic/pharmaceutical groups. In addition he leads a team of Scientists at various levels in their careers and is working with other groups at Complete Genomics to develop LFR into a product offering.

5. Careers in Academia and Grants Management

This workshop will focus on professionals that will discuss their career paths in academia and grants management. In academia, the ability to write grants and have money for your research is critical for a successful career. We will have an overview of options of grants for young investigators and the components of grants management. We will also highlight on how to develop research ideas, write clear and persuasive grant applications, and how to successfully communicate scientific data and ideas. Finally we will discuss a career in a small liberal arts & science college with the main focus on teaching.

Nancy C. Lohrey, M.S., M.T.-American Society Clinical Pathology

Nancy is the Program Director for the NIH/NCI Pathway to Independence (K99/R00), and the congressionally mandated NIH/NCI T32 Ruth L. Kirschstein National Research Service Awards (NRSA) Institutional Training Programs. Ms. Lohrey is the NIH Liaison for the NIH/NCI Loan Repayment Program. She has administered several other training mechanism in the NCI/OD/CCT/CTB: the Howard Temin K01 Awards, postdoctoral F32 Ruth L. Kirschstein National Research Service Awards (NRSA), the K08 Clinical Scientist Mentored Career Development grants, the K05 Established Investigator Award in Cancer Prevention, Control, Behavioral, and Population Sciences Research, and the F33 NIH National Research Service Award for Senior Fellows.

She received her B.S. in Biology and Chemistry at Ohio State University, Columbus, Ohio and her M.S. in the Microbiology and Biochemistry at Southern Illinois University (Edwardsville). She then completed a year's residency as a Medical Technologist and passed the national MT-American Society of Certified Pathologists exam. She spent another year working with the equine retrovirus, EIAV (Equine Infectious Anemia Virus) at the Veterinary Research Institute at Louisiana State University in Baton Rouge, Louisiana. She then participated in a PhD scholarship program at the University of Alberta Medical School, University of Alberta, in Canada. After completing two years of her studies (All-But-Thesis), she joined the NCI. She spent five years with RAEB (Research, Analysis and Evaluation Branch) of the Division Extramural Activities, NCI, after having completed thirteen years of NCI Intramural Research in the Laboratory of Leukocyte Biology at the National Cancer Institute in Frederick in the laboratory of Francis Ruscetti. Hematopoietic stem cell therapeutics and AIDS research were her main areas of study.

Margarita (Magui) Cardona, M.S., C.R.A.

Magui Cardona has spent the majority of her professional career in research administration. She is currently the Director of Sponsored Research at the University of Baltimore. In that capacity, she coordinates fiscal and program management for all externally funded research and scholarly activities at UB. She also oversees the activities of the Institutional Review Board, and all internal and external reporting related to sponsored research funding. Prior to UB, she worked as Grants Manager and later as a Senior Grants and Contracts Manager in the Office of Sponsored Programs at the University of Maryland, Baltimore County. She also held two contractual positions at the National Air and Space Administration. At NASA Headquarters she worked as a Space Grant Administrative Fellow in the Office of Education, managing 52 grants and 20 cooperative agreements for the agency. She also worked at Goddard Space Flight Center as a Louis Stokes Administrative Fellow in the Minority University Programs Office, providing technical assistance to minority serving institutions in how to obtain NASA funding. Before NASA, she worked at the University of Puerto Rico as a Program Coordinator for the Puerto Rico Space Grant and

EPSCoR programs. She holds a BS in Chemistry from the University of Puerto Rico and two MS degrees in Chemistry (UNC-Chapel Hill) and Materials Science (UW-Madison). Magui is also a Certified Research Administrator and serves as Secretary for Region II of the National Council of University Research Administrators. She is currently pursuing a doctorate in Public Policy at UMBC.

Ram Savan, Ph.D.

Dr. Ram Savan received his Ph.D in 2004 from United Graduate School of Agriculture Sciences, Kagoshima University, Japan under Dr. Masahiro Sakai. Dr. Savan was a visiting fellow in the Laboratory of Experimental Immunology, Cancer and Inflammation Program, National Cancer Institute (NCI), NIH in Dr. Howard Young's laboratory. Currently, he is a tenure track Assistant Professor in the Department of Immunology, University of Washington, Seattle. His research is focused on post-transcriptional regulation of immune genes. During his post-doctoral work, he defined a novel role for miRNAs in stabilizing interferon gamma which represents a major advance in the field of post transcriptional regulation. He also identified the microRNA controlling HLA-C gene expression in collaboration with Dr. Mary Carrington's laboratory. This work has implications for the differential susceptibility of individuals to HIV. The other area of his research interest was defining the importance of IL-22 receptor expression in the pathogenesis of ALK+ anaplastic large cell lymphoma which is a pediatric lymphoma. He has been a recipient of the Milstein Young investigator award, from the International Society for Interferon and Cytokine Research. He is a two time recipient of National Cancer Institute-Directors Innovation award in 2009 and 2010.

Ancha Baranova, Ph.D.

Dr. Ancha Baranova is an Associate Professor in the School of Systems Biology, College of Science, George Mason University in Fairfax, Virginia, USA. Dr. Baranova graduated from Moscow State University (Moscow, Russia) in 1995 with an MS in Biochemistry/Virology then continued to her PhD in Virology (1998). Dr. Baranova relocated to the United States in 2002 after she joined the Molecular and Microbiology Department (George Mason University, Fairfax, VA) as an Assistant Professor, and received her tenure in 2007. Since 2005 Dr. Baranova also serves as Assistant Director of the Center for Study of the Genomics of Liver Disease in the College of Sciences, George Mason University.

Dr. Baranova's major academic contributions are in the field of functional genomics, with emphasis on cancer and metabolic syndrome-related disorders. Since 1992 she has participated in international collaborations aimed at the positional cloning of the tumor suppressor gene rearranged in B-cell chronic lymphocytic leukemia. A significant part of Dr. Baranova's efforts is dedicated to in silico analysis of the publicly available genomics and proteomics database. Dr. Baranova sees these databases as treasure troves full of diamonds waiting to be unearthed and cut. Based on a computational prediction made by Dr. Panchin (Institute of Problems of Information Transmission, Russian Academy of Science), Dr. Baranova discovered and studied pannexins, a novel class of gap junction proteins present in both chordate and invertebrate genomes. The past few years have added a novel focus to Dr. Baranova's research as she has gotten involved in collaboration with the Center for Liver Diseases at Inova Fairfax Hospital, Northern Virginia. In this collaboration, Dr. Baranova has published a number of papers uncovering molecular pathways altered in the pathogenesis of Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis in morbidly obese patients and developed an ELISA-based diagnostic approach for their non-invasive detection.

Craig Laufer, Ph.D.

Craig Laufer is Professor of Biology at Hood College. He earned his B.S. in biochemistry and zoology from the University of Maryland, College Park in 1979 and Ph.D. in biochemistry from Kent State University in 1984 followed by post-doctoral studies at the University of Maryland, Baltimore County. He has been in the Department of Biology at Hood College since 1988. Dr. Laufer teaches courses in introductory biology, microbiology, protein biochemistry and prokaryotic genetics. His recent research interests include applying directed evolution approaches toward engineering enzymes for use in biomass to fuel applications. Specifically he has been engineering thermal stability and greater activity in pectin methylesterases and pectin acetylesterases.

6. Communicating Science: Getting the Message Out

Scientists traditionally share their research findings with their peers and funding agencies customarily through peer-reviewed publications, conferences, progress reports and other conventional avenues. However, the advent of the modern communication has dramatically widened the target audience for scientists, with research communication also requiring direct interaction with the public, media, policymakers and other stakeholders. The public's arguable perception is that many scientists are not socially savvy, lacking the necessary skills to communicate effectively. This interactive skill-building workshop is designed to explain the importance of translating your work for greater personal and professional rewards, by engaging diverse audience through effective communication. The goal of this workshop is to assist scientists to bridge the widening gap between them and the world-at-large, through valuable communication techniques that will help them to get the message out.

Evonne Kaplan-Liss M.D., M.P.H., F.A.A.P.

Dr. Evonne Kaplan-Liss is an Assistant Professor of preventive medicine and pediatrics in the School of Medicine at the State University of New York at Stony Brook. She is a core faculty member in Stony Brook's Graduate Program in Public Health, where her main initiative is to work as Director of the Advanced Graduate Certificate in Health Communications. This is a collaboration between the University's Graduate Program in Public Health and School of Journalism.

Dr. Kaplan-Liss is part of the steering committee and also a lecturer for Stony Brook's Center for Communicating Science. There she works to enhance understanding of science by helping train the next generation of scientists and health professionals to communicate more effectively with the public, public officials, the media, and others outside their own discipline. She is also the managing editor of a textbook in disease prevention, entitled Health Promotion and Disease Prevention in Clinical Practice, 2nd Edition. Before joining Stony Brook, she was a pediatrician in a thriving family practice in New York.

Dr. Kaplan-Liss' journalism career began at her first job as a researcher for Ted Koppel's Nightline for ABC News. She left Nightline to advance her interest in medical journalism, working as an associate producer and then segment producer on medical news for syndicated programs Instant Recall and First Look.

She graduated from the Mount Sinai School of Medicine in Manhattan in 1997 and then completed a residency in Pediatrics at North Shore University Hospital in Long Island. After working in private practice, Dr. Kaplan-Liss completed a residency in preventive medicine at Stony Brook while receiving a Masters in Public Health from Columbia University's Mailman School of Public Health.

7. Navigating Work Relationships: Recognizing Conflict Pitfalls and Opportunities

It can often be challenging for trainees to figure out how to negotiate with their supervisors and talk about difficult issues in the lab while preserving their working relationships and protecting their careers. This can be even more challenging when people work in diverse environments with people from other cultures. This workshop will focus on ways to negotiate that build long-lasting solutions, strengthen relationships and use positive interpersonal skills.

Attendees will:

- Gain awareness of their own preferred conflict style and how it might affect the way they deal with difficult situations
- Explore about the role of emotions in conflict
- Learn how to identify and address the concerns of others in order to find mutually satisfying solutions to problems
- Think about how cultural differences can impact communications
- Learn how to prepare for negotiations and difficult conversations
- Practice active listening and communication skills

Howard Gadlin, Ph.D.

Howard Gadlin has been Ombudsman and Director of the Center for Cooperative Resolution at the National Institutes of Health since the beginning of 1999. From 1992 through 1998 he was University Ombudsperson at UCLA. He was also director of the UCLA Conflict Mediation Program and co-director of the Center for the Study and Resolution of Interethnic/Interracial Conflict. While in Los Angeles, Dr. Gadlin served as consulting Ombudsman to the Los Angeles County Museum of Art. Prior to coming to UCLA, Dr. Gadlin was Ombudsperson and Professor of Psychology at the University of Massachusetts, Amherst. At present Dr. Gadlin is studying the dynamics of scientific teams and collaborations and developing new approaches to addressing conflicts among scientists An experienced mediator, trainer, and consultant, Dr. Gadlin has years of experience working with conflicts related to race, ethnicity and gender, including sexual harassment. Currently he is developing new approaches to addressing conflicts among scientists. He is often called in as a consultant/mediator in "intractable" disputes. Dr. Gadlin has designed and conducted training programs internationally in dispute resolution, sexual harassment and multicultural conflict.

Dr. Gadlin is past President of the University and College Ombuds Association (UCOA) and of The Ombudsman Association (TOA). For three years, he was chair of the Ethics Committee of the Society of Professionals in Dispute Resolution. He also served 5 years as Chair of the Coalition of Federal Ombudsmen. He is currently the chairperson of the federal Inter-agency Alternative Dispute resolution Working Group steering committee. Dr. Gadlin is the author of, among other writings, "Bargaining in the Shadow of Management: Integrated Conflict Management Systems," "Conflict, Cultural Differences, and the Culture of Racism," and "Mediating Sexual Harassment." He is the co-author of "Neutrality: What an organizational ombudsperson might want to know" and "Conflict Resolution and Systemic Change." Most recently he has co-authored "Collaboration & Team Science: A Field Guide."

Linda Myers, J.D.

Linda Myers is an Associate Ombudsman at the National Institutes of Health, Office of the Ombudsman. She has worked as an Alternative Dispute Resolution program manager, mediator, and facilitator for the federal government since 1994. Her ADR and mediation background includes work in equal employment opportunity cases, prohibited personnel practices complaints, including whistleblower reprisal, labor-management disputes, and organizational conflict resolution. Linda has extensive experience in conducting training and education programs in interest-based negotiation; communication and conflict resolution; and providing advice and guidance to organizations on the development of workplace ADR programs. Immediately prior to joining the NIH Office of the Ombudsman, Linda served as Deputy Director of the U.S. Army ADR Program in the Office of the Army General Counsel. While at the Army ADR Program, Linda designed and delivered ADR and negotiation training and education programs at Army installations worldwide. Linda is a graduate of Loyola University of Chicago and received her J.D. from Chicago-Kent College of Law

Oral Presentation and Poster Abstracts

Immunology I

Oral Presentation

FBXW7alpha attenuates inflammatory signaling by suppressing expression of Cebpd and its target gene Tlr4

Kuppusamy Balamurugan[1], Kimberly D. Klarmann[2], Youhong Zhang[1], Vincenzo Coppola[3], Glenn H. Summers[4], Thierry Roger[5], Jonathan R. Keller[2], Shikha Sharan[1], and Esta Sterneck[1]

[1]Laboratory of Cell and Developmental Signaling; [2]Laboratory of Cancer Prevention, CCR, NCI-Frederick; [3]Ohio State University, Columbus, OH; [4]Laboratory of Animal Sciences Program, SAIC, CCR, NCI-Frederick; [5]Infectious Diseases Service, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland

The Toll-like receptor 4 (TLR4) plays an important role in initiating innate immune responses against certain pathogens. TLR4 signaling activates NF-kB transcription factors that induce inflammatory response genes such as the transcription factor CCAAT/enhancer binding protein delta (CEBPD). CEBPD amplifies LPS signaling and is essential for survival of infection by gram-negative bacteria (Litvak et al, 2009). In the present study, we identified TLR4 as a direct target gene of CEBPD. The basal levels of CEBPD support TLR4 expression in macrophages/monocytes and mammary tumor cells. Therefore, CEBPD is not simply a downstream effector of LPS-signaling but also necessary upstream of LPS. These data also call for a re-evaluation of the precise role of CEBPD downstream of TLR4. Previously, we reported that CEBPD promotes hypoxia-inducible factor-1alpha (HIF-1alpha) expression and hypoxia adaptation in mammary tumor cells by inhibiting expression of F-box and WD repeat domain containing protein 7alpha (FBXW7alpha), which correlates with metastatic progression of tumors (Balamurugan et al, 2010). Here we show that FBXW7alpha in turn suppresses CEBPD gene expression and thereby attenuates the inflammatory response. Ectopic FBXW7alpha reduced CEBPD expression and abolished LPS responses in macrophages, whereas FBXW7 depletion augmented CEBPD/TLR4-mediated signaling. In mice, in vivo RNAi against Fbxw7a was sufficient to induce pro-inflammatory factors in peritoneal cells. Inflammation is strongly associated with tumor progression. Cebpd null mammary tumors showed higher levels of Fbxw7alpha and diminished Tlr4 expression and reduced pro-inflammatory markers, which is in line with the reduced metastatic potential of Cebpd deficient tumors. Taken together, these findings revise our understanding of the role of CEBPD in TLR4 signaling, and uncover a novel function for FBXW7alpha as an attenuator of inflammatory responses. We propose that fine tuning of the negative feedback loop between CEBPD and FBXW7 may be critical to control the magnitude of the inflammatory response.

Oral Presentation

HN3: a human single-domain monoclonal antibody binds cell surface-associated glypican-3 and inhibits hepatocellular carcinoma cell proliferation

Mingqian Feng[1], Heungnam Kim[1], Ruoqi Wang[1], Weizao Chen[2], Yao-Gao Man[3], Dimiter S. Dimitrov[2], and Mitchell Ho[1]*

[1]Antibody Therapy Section, LMB, CCR, NCI, Bethesda, MD; [2]Protein Interaction Group, CCR, NCI-Frederick, Frederick, MD; 3Armed Forces Institute of Pathology and American Registry of Pathology, DC

Liver cancer is the fifth most common malignant cancer worldwide. Hepatocellular carcinoma (HCC) accounts for about 75% of primary liver cancer cases. Currently, surgery is the standard treatment for liver cancer. Liver cancer does not respond to most chemotherapy drugs. There is an urgent need to develop new drugs with different mechanisms of action. Monoclonal antibody therapy represents a promising approach, but it remains a challenge mainly due to lack of tumor-specific targets. Glypican-3 (GPC3) has recently emerged as an attractive candidate for liver cancer therapy given that it is highly expressed in HCC. The biological function of GPC3 is largely unknown. Our recent work was one of the first to investigate the role of GPC3 in HCC cell proliferation [Int J Cancer. 2011;128:2246-7]. We produced a recombinant soluble GPC3 protein without the GPI anchor (sGPC3) and discovered that sGPC3, functioning as a dominant-negative form, directly inhibited the growth of GPC3-expressing HCC in vitro. Based on this novel observation (and that of others), we hypothesize that blocking GPC3 signaling may represent a novel therapeutic approach to inhibit HCC growth for cancer therapy. Here, we have applied our expertise in phage display antibody technology and identified a high affinity engineered human antibody domain (named HN3). HN3 binds cancer cell surface-associated GPC3 molecules with sub-nanomolar affinity. HN3 recognizes a novel conformation epitope in the core protein of GPC3. Furthermore, HN3 can inhibit proliferation of HCC cells and induce apoptosis. Our work shows for the first time that it is possible to inhibit HCC cell proliferation by a GPC3 inhibitor. HN3 has the potential as a novel therapeutic monoclonal antibody for liver cancer therapy.

Oral Presentation

Interleukin (IL)-1beta controls the Dectin-1mediated programming of human dendritic cells via IkappaB-zeta

Marco Cardone[1], Amiran K. Dzutsev[2], Hongchuan Li[2], Franca Gerosa[3], Lisa Provezza[3], Elena Riboldi[1], Mark D. Wewers[4], Charles A. Stewart[1], Folkert Steinhagen[1], Selinda J. Orr[1], Daniel W. McVicar[1], Stephen K. Anderson2, Romina Goldszmid[1], Giorgio Trinchieri[1], and Lyudmila Lyakh[1]

[1]Cancer and Inflammation Program, Laboratory of Experimental Immunology, NCI-Frederick, Frederick, MD; [2]Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [3]Department of Pathology, Section of Immunology, University of Verona, Verona, Italy; [4]The Ohio State University, Columbus, OH

Human dendritic cells (DC) are antigenpresenting cells (APC) capable of polarizing helper T (TH) lymphocytes into TH1, TH2, TH-17, and the recently described TH-22 cells. Pattern recognition receptors (PRRs) mediated programming of DC controls TH cell polarization, and it is essential to ensure an adequate protection of the host. We have previously shown that human monocyte-derived DC (mono-DC) produce IL-1beta, IL-6, and IL-23 after stimulation with the Dectin-1 ligand beta-glucan, a component of cell wall of fungi and yeast, including Candida Albicans. Dectin-1mediated programming of mono-DC induced the development of TH-17 cells and IL-1beta was found to play a central role in this process. To dissect the molecular mechanisms controlling the production of TH-17promoting cytokines in DC, we sought to determine if and to what extent endogenously produced IL-1 and TNF play a role in this process. We found that both beta-glucan and LPSstimulated mono-DC produce TNF with similar kinetics but only beta-glucan induces high levels of bioactive IL-1beta, in an inflammasomedependent manner. At the same time, we observed that IL-1 and TNF were produced at earlier time points compared to IL-6, IL-23, IL-12B, and IL-10. We next examined whether these early cytokines were responsible for driving the late inflammatory response and subsequent the DC programming. We found that a positive feedback through autocrine IL-1beta and the IL-1 inducible nuclear factor IkappaB-zeta, are distinctly required for the transcription of late response genes in beta-glucanactivated human mono-DC. These genes include those encoding the TH-17polarizing cytokines IL-6 and IL-23, as well as IL-10. Thus, our findings show that human mono-DCderived IL-1beta favors the induction of TH-17 responses by acting both on APC and on responding CD4+ T cells and strengthen the idea that IL-1 antagonism may efficiently prevent or ameliorate TH-17 related diseases.

Oral Presentation

Tumor cells contain veto factors limiting immunogenicity of sterile necrosis and controlling CD8+ T cell activation

Jaba Gamrekelashvili, Tamar Kapanadze, Firouzeh Korangy, and Tim F. Greten

Medical Oncology Branch, NCI, NIH

Generation of CD8+ T cell dependent immune responses is important for anti-tumor immunity and for successful anti-cancer therapy. Therefore, dissecting mechanisms regulating the function of these cells as a consequence of tumor cell death is of great interest. We have investigated cellular and molecular events responsible for T cell activation during sterile necrotic cell death in the absence of Pathogen Associated Molecular Patterns (PAMPs). Sterile necrosis was induced by 3 freeze/thaw cycles in Ovalbumin (OVA) expressing, MHC class I negative cells. Sterile necrotic cells failed to stimulate CD8+ T cell response when incubated with dendritic cells (DCs) in vitro. In contrast, activation of 3xF/T necrotic cells induced proliferation and IFN-γ secretion by antigen-specific CD8+ T cells in vitro. Injection of immunogenic necrotic cells in vivo or adoptive transfer of CD11c+ splenic DCs loaded with immunogenic necrotic cells led to efficient adaptive immune response and protection from tumor challenge. In contrast non-immunogenic necrotic (3xF/T) tumor cells failed to induce OVA specific CD8+ T cell dependent immunity. We hypothesized and now show that 3xF/T tumor cells contain certain factors, which limit antigen processing/presentation and therefore make necrotic cells non-immunogenic. We refer to these as veto factors. Using classical chromatography and mass-spectrometry approaches, we have identified two different veto factors. When released from 3xF/T necrotic cells they can abort priming of CD8+ T cells. Exogenous veto factors drastically reduced proliferation and IFN-γ secretion from antigen-specific T cells in vitro. In vivo co-administration of exogenous veto factors together with immunogenic necrotic cells precluded the generation of antigen-specific immune response. These results demonstrate that impairment of immunogenicity of dead cells in conditions when cellular integrity is lost and intracellular content is released, is a new mechanism to control adaptive immune responses and these findings will help in designing better cancer vaccines.

Oral Presentation

Co-immunization with HIV env DNA and Protein Elicit Both Strong Cellular and Humoral Immune Responses

Jinyao Li[1], Antonio Valentin[1], Viraj Kulkarni[2], Candido Alicea[2], Rachel Beach[1], Margherita Rosati[1], Rashmi Jalah[2], Steven Reed[3], Barbara K. Felber[2], and George N. Pavlakis[1]

[1]Human Retrovirus Section, [2]Human Retrovirus Pathogenesis Section, Vaccine Branch, CCR, NCI-Frederick; [3]Infectious Disease Research Institute, Seattle

Background: An efficacious prophylactic HIV-1 vaccine should elicit both strong cellular and humoral immune responses. We recently demonstrated that potent, long-lasting HIV-1 Env-specific cell-mediated immune responses could be elicited in rhesus macaques and mice using plasmid encoding env DNA as the immunogen. In this study, we examine a vaccine platform combining DNA and protein co-immunization to generate both strong cellular and humoral immune responses. Methods: Mice or macaques were immunized with HIV env gp120 DNA vaccine and/or purified gp120 protein from clade B or clade C isolates. For mice, three groups (n=5) were immunized twice at 4 weeks interval with DNA only, protein only formulated in EM005 adjuvant, and DNA&protein/EM005. Macaques were immunized twice at 4 weeks interval with DNA only, DNA&protein, DNA&protein/EM005. Results: DNA&protein co-immunization enhanced the humoral immune responses compared with DNA only and protein only in mice. DNA&protein co-immunization generated similar level of cellular immune responses as DNA only but the level was much higher than those of the protein only group in mice. This strategy also elicited higher humoral immune responses than DNA only in macaques. The presence of the EM005 adjuvant further enhanced the Ab responses in mice and macaques. These responses were correlated with the up-regulated activation of dendritic cells by EM005. Conclusion: The strategy of DNA and protein co-immunization has good potential for development as an improved prophylactic HIV-1 vaccine.

#01

Chimeric Antigen Receptor therapy against GD2-expressing pediatric tumors

William Babbitt[1] Rimas Orentas[2] and Crystal Mackall[2]**

[1]University of California, San Francisco; [2]Pediatric Oncology Branch, NCI

Continued poor prognosis of pediatric solid tumors has generated widespread research into new anti-cancer strategies. One such approach is to target unique or over-expressed cell surface components by immunotherpies such as antibodies or cytotoxic T-cells with target specificity. Disialoganglioside (GD2) is highly expressed on several pediatric tumors, including neuroblastomas and some soft-tissue sarcomas, with normal tissue expression limited in small amounts to peripheral nerves, making it a promising anti-cancer target. Immunotherapy for GD2-expressing tumors has shown efficacy in early clinical trials of anti-GD2 monoclonal antibodies (mAb) in the context of bone-marrow transplant. Chimeric antigen receptors (CARs) are single chain variable fragment (scFv) sequences derived from mAbs linked to T-cell specific intracellular activating motifs that have been transduced into T-cells using a viral vector. This technology allows one to harness the specificity of a monoclonal antibody with the potency and longevity of a T-cell. We sought to develop an anti-GD2 CAR and test its efficacy in both in vitro and in vivo models. CARs to GD2 were created using retroviral transduction of sequences derived from the 14G2a mAb to GD2. In vitro data demonstrates enhanced killing of GD2-expressing neuroblastoma, rhabdomyosarcoma, and osteosarcoma cell lines over that of non-expressing lines. In vivo data in a xenogeneic mouse model did not initially show inhibition of tumor growth by CARs as compared to mock T-cells. Preliminary data suggests that this relates to poor CAR trafficking to the tumor site. In 5 out of 10 mice, spleen analysis showed persistence and expansion of CAR-expressing T-cells, while tumors contained virtually none. Current studies are underway to better understand the factors regulating trafficking of CAR-transduced T-cells to sites of solid pediatric tumors and to overcome this limitation.

#02

Myeloid cell migration plays a critical role in solid tumor regression induced by the dipeptidyl peptidase inhibitor 4175

Brynn Duncan[1], Najat Bouchkouj[1], Steven Highfill[2], William Bachovchin3, Terry Fry[1]

[1]Blood and Marrow Transplant Section, Pediatric Oncology Branch, CCR, NCI; [2]Immunology Section, Pediatric Oncology Branch, CCR, NCI; [3]Department of Biochemistry, Tufts University School of Medicine, Boston, MA

We have previously shown that the novel compound 4175, a second-generation pan-inhibitor of dipeptidyl peptidases (DPPs), induces complete regression of established solid tumors in mice. This effect is both T cell and DC dependent, but is also associated with changes in myeloid cell subsets as well. The frequency of myeloid derived suppressor cells (MDSCs) in the tumors and spleens of 4175-treated mice varied significantly from saline-treated controls. As the dose of 4175 increased from 5ug to 200ug per dose, there were fewer CD11b+IL-4Rα+Ly6C+Ly6Glo cells observed in the tumor but more in the spleen, suggesting these monocytic MDSCs were not migrating to the tumor microenvironment. 4175 treatment not only reduced this suppressive population within the tumor, but it also induced migration of myeloid DCs to the tumor-draining lymph node (TDLN). More CD11c+CD11b+ cells were observed in TDLNs of mice treated with doses of 4175 capable of eradicating tumor. Finally, depleting Gr1+ cells reduced the efficacy of the first-generation DPP inhibitor PT-100, indicating that myeloid cells are required for this anti-tumor activity. Chemokine gradients are largely responsible for immune cell migration, and DPPs are known to modulate activity of various cytokines and chemokines. We hypothesized that inhibition of DPPs would result in a shift in the levels of active chemokines in the periphery, perhaps abrogating the gradient needed to recruit MDSCs to the tumor and possibly intensifying the gradient needed to recruit myeloid DCs to the TDLN. Using PT-100 in plt/plt mice and in CCR7-/- bone marrow chimeras, we observed a loss of drug-induced anti-tumor activity, suggesting the CCR7-CCL19/21 axis plays a central role in the mechanism of these inhibitors.

#03

Generation and optimization of a chimeric antigen receptor against human CD22: A new immunotherapeutic agent for adoptive immunotherapy

Waleed Haso[1], Daniel Lee, III[1], Richard Morgan[2], Ira Pastan[3], Crystal Mackall[1], Rimas J. Orentas[1]

[1]Pediatric Oncology Branch, CCR, NCI; [2]Surgery Branch, CCR,NCI; Laboratory of Molecular Biology, CCR, NCI, Bethesda, MD

CD22 is expressed on a number of hematologic malignancies. A recombinant immunotoxin composed of an anti-CD22 Fv fused to a 38 kDa fragment of Pseudomonas exotoxin A (Moxetumomab pasudodtox, HA22) is currently being evaluated in the treatment of B cell malignancies. We used the Fv sequence to construct a series of chimeric antigen receptors (CARs) to determine the optimal affinity, domain structure, and signaling required for optimal anti-leukemic activity. The original anti-CD22 binding domain, BL22, or a newer generation high-affinity domain, HA22, were fused to transmembrane and signaling sequences derived from the TCR zeta-chain, CD28, and CD137. In some constructs we extended the binding domain away from the membrane using constant domains from IgG (CH2CH3). Retroviral gene vectors were used to transduce activated primary T cells with CAR constructs. We found: HA22 Fv, 2 as opposed to 3 signaling motifs, and non-CH2CH3 containing CARs were superior in short-term in vitro tumor cell cytolysis assays, indicating that Fv affinity, signaling, and 3-D structure of the CAR all impact the anti-leukemic effectiveness of CARs. Finally, direct comparison to CD19-specific CAR showed an equivalent or superior killing activity in 2 out of 3 ALL lines tested. Using Scatchard analysis to define the number of CD22 molecules on the surface, the ALL line with the lowest number of CD22 molecules was also the least able to be killed by CAR-transduced T cells. The superior lytic activity of CD19-CAR for this single cell line was due to preservation of high levels of CD19 at the cell surface. We are currently testing the in vivo activity of anti-CD22 CAR in an immunodeficient mouse model bearing human cell lines and primary patient-derived ALL. Our results indicate that CD22-CAR should be developed for the immunotherapy of CD22+ malignancies.

#04

Interaction between the normal B cell bone marrow niche and pre-B cell acute lymphoblastic leukemia

Elizabeth D. Hicks[1], and Terry Fry[2]

[1]Clinical Research Training Program, Office of Clinical Research Training and Medical Education, CC, NIH; [2]Pediatric Oncology Branch, CCR, NCI, NIH

While advances in chemotherapy and bone marrow transplant have dramatically improved survival for children with acute lymphoblastic leukemia, it is still responsible for the highest number of cancer deaths in children. A better understanding of leukemia in the bone marrow microenvironment could lead to targeted therapies that disrupt this environment. We sought to characterize the bone marrow niche of leukemia, and what signals may be important for survival and disease progression. We initially injected C57Bl/6 mice with a preB-I cell acute lymphoblastic leukemia line from an E2a-PBX1 transgenic mouse. We harvested bone marrow from these mice and used flow cytometry to show that as the leukemia progressed, the frequency of proB cells (B220+CD43+BP1-CD25-) increased while the frequency of preB-I (B220+CD43+BP1+CD25-) and early preB-II (B220+CD43+BP1+CD25+) cells decreased. This could be explained by displacement of normal preB-I cells in the bone marrow by leukemic cells, causing a block at this stage of B cell development and an increase in proB cells. These results support the hypothesis that the leukemia may be usurping the non-malignant preB-I cell niche. Next, we looked at what signals the leukemia may be getting from this niche. Many human ALL lines have gain-of-function mutations in the genes for TSLP and IL-7 receptors, implicating these cytokines in leukemia survival. We found that E2aPBX expresses all components of both the IL-7 receptor and the TSLP receptor: IL-7Ra, gamma-C receptor, and TSLPR. When stimulated with either IL-7 or TSLP in vitro, E2aPBX phosphorylates STAT5, as shown by intracellular flow cytometry. STAT5 phosphorylation occurs downstream of both IL-7 and TSLP signaling. Further, E2aPBX1 surface expression of IL-7Ra decreases when stimulated with IL-7 in vitro. Conversely, surface expression of TSLPR decreases in response to TSLP stimulation. These results demonstrate that both the IL-7 and TSLP receptor complexes are likely to be functional.

#05

An in vivo requirement for peripheral IL-7 in nave T cell homeostasis

Hilary R. Keller[1], Megan Luckey[1], Grace Y. Kim[1,2], and Jung-Hyun Park[1]

[1]Experimental Immunology Branch, CCR, NCI; [2]Johns Hopkins University School of Medicine, Baltimore, MD

Interleukin-7 (IL-7) signaling is essential for T cell development in the thymus and also for peripheral T cell homeostasis. So far, the role of peripheral IL-7 distinct from its role in thymopoiesis has been difficult to assess. Thus, we generated a mouse model in which IL-7 signaling is limited to the thymus and is absent in the periphery. The mouse, referred to as K7, was generated through mating of a proximal lck enhancer driven IL-7 transgene onto an IL-7 deficient background. The K7 mice, therefore, express IL-7 in thymocytes but not in mature T cells. Lymph node T cell numbers in K7 mice were dramatically reduced, indicating a critical role of IL-7 in T cell survival. However, K7 mice still retained a small population of T cells which were mostly of a CD44 low, nave phenotype. To address whether these cells survive without IL-7 or whether they are recent thymic emigrants, we employed T cell receptor excision circle (TREC) analysis. Thymocytes and lymph node T cells from wildtype and K7 mice of differing ages were analyzed for TREC numbers, and we found that TREC numbers in lymph node T cells of wildtype mice decreased with age. Importantly, in peripheral T cells of K7 mice, we did not find any significant difference to wildtype T cells. To further assess the identity of peripheral K7 cells, we thymectomized K7 mice and analyzed the peripheral T cell compartment 6 weeks after operation. Remarkably, most of the nave T cells had disappeared, and only very small numbers of CD44 high memory cells remained. Thus, the peripheral T cells in the K7 mice survive for a short term without IL-7, further indicating that IL-7 is necessary for long term maintenance and homeostasis of T cells.

#06

The Transmembrane Domain of MHC II Regulates Lipid Raft Microdomain association.

Sanjay Khandelwal and Paul A Roche

Experimental Immunology Branch, CCR, NCI

MHC II is present in cholesterol-dependent lipid raft plasma membrane microdomains, and raft association is important for MHC II- dependent activation of CD4 T cells by dendritic cells (DCs). Until now it is not clear what regulates MHC II lipid raft association. In the present study we attempt to define the signals present in the MHC II molecule that regulate its association with lipid rafts. To determine the role of MHC II cytoplasmic domain in lipid raft association we expressed tail less MHC II alpha and beta chain in DCs obtained from MHC II-null mice by lentiviral transduction. Our results show that MHC II cytoplasmic tail does not have significant role in MHC II lipid raft association. Palmitoylation of cysteine residues is a well-known modification that often results in lipid raft association of TM proteins and each MHC II I-Ab subunit contains a TM cysteine. To our surprise, replacement of the MHC II alpha and beta chain TM domain cysteine with serine actually enhanced MHC-II raft localization. We also examined the role of the MHC II transmembrane (TM) domains in lipid raft association by replacing the TM domain of MHC II alpha and beta chain with a non-raft protein ICAM 1 TM domain. These chimeric MHC II alpha and beta chains assemble into MHC II alpha beta dimers, are expressed on the surface of DCs, and are functional in alloreactivity assays. Membrane fractionation revealed that the lipid raft association of these chimeric MHC II is reduced 5 fold. Thus we have identified the TM domain of MHC II as a prominent regulator of lipid raft association.

#07

Deconstructing Ras signaling in the thymus

Robert L. Kortum[1], Connie L. Sommers[1], John M. Pinski[1], Clayton P. Alexander[1], Robert K. Merrill[1], Wenmei Li[1], Paul E. Love[2], and Lawrence E. Samelson[1]

[1]Laboratory of Cellular and Molecular Biology, NCI; [2]Laboratory of Mammalian Genes and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD

Thymocytes must transit at least two distinct developmental checkpoints, governed by signals that emanate from either the pre-T cell receptor (pre-TCR) or the TCR to the small G protein Ras, before emerging as functional T lymphocytes. Ras is activated in thymocytes by two families of Ras guanine exchange factors (RasGEFs): RasGRP1 and Son of Sevenless (Sos1 and Sos2). Recent studies have shown a role for Sos1, but not RasGRP1, at the pre-TCR checkpoint. At the second checkpoint, the quality of signaling through the TCR is interrogated to ensure the production of an appropriate T cell repertoire. Although RasGRP1 is the only confirmed RasGEF required at the TCR checkpoint, current models suggest that the intensity and character of Ras activation, facilitated by both Sos and RasGRP1, will govern the boundary between survival (positive selection) and death (negative selection) at this stage. Using mouse models, we have assessed the independent and combined roles for the RasGEFs Sos1, Sos2, and RasGRP1 during thymocyte development. Although Sos1 was the dominant RasGEF at the pre-TCR checkpoint, combined Sos1/RasGRP1 deletion was required to effectively block development at this stage. Conversely, while RasGRP1 deletion efficiently blocked positive selection, combined RasGRP1/Sos1 deletion was required to block negative selection. This functional redundancy in RasGEFs during negative selection may act as a failsafe mechanism ensuring appropriate central tolerance.

#08

AKT Inhibition Uncouples Proliferation and Differentiation in Stimulated CD8+ T-Cells

Anthony Leonardi and Joseph Crompton

Surgery Branch, CCR, NCI

Expanding T cell populations via concurrent TCR stimulation and IL-2 signaling is a well characterized and utilized method for generating expanded pools of effector CD8+ T cells. IL-2 signaling and TCR stimulation have been shown to cause increased AKT signaling that promotes effector differentiation. Here we show that inhibiting the AKT pathway with dramatically hinders the differentiation process in vitro while paradoxically increasing culture expansion and T cell yield. This capability presents immunotherapy researchers and clinicians a valuable tool in aiding the expansion of a culture and preventing premature in vitro terminal differentiation of cytotoxic lymphocytes. We show treatment efficacy in a mouse model of Adoptive Cell Transfer for the treatment of B16 melanoma in BL6 mice by transgenic CD8+ T cells expressing the Pmel TCR specific to the GP100 antigen restricted by H2Db.

#09

IFN-gamma is the primary cause of aplastic anemia not autoreactive T cells

Fanching Lin, Michael W. Macfarlane, Bahara Saleh, Deborah L. Hodge and Howard A. Young

Laboratory of Experimental Immunology, Cancer and Inflammation Program, NCI, Frederick

Aplastic anemia (AA) is characterized by hypocellular marrow and peripheral pancytopenia. Although cases of AA caused by environmental factors have been reported, the etiology of AA is largely unknown. Since IFN-γ and T-bet can be detected in AA patients' T cells, it was believed that autoreactive T lymphocytes played a major role in destroying the hematopoietic stem cells (HSCs) in bone marrow (BM). Thus, AA has been treated as an autoimmune disease. We have observed AA-like symptoms in our IFN-γ AU-rich element (ARE) knock-out (KO) mice. These mice have a targeted deletion in the 3'untranslated region of the IFN-γ mRNA. The deletion increases the half-life of IFN-γ mRNA. Thus, these mice constitutively express low level of IFN-γ under normal condition. In this study, we used IFN-γ ARE KO mice as an animal model to understand the etiology of AA. These mice exhibited signs of immunodeficiency when their immune organs were examined. Also, T cells from these mice failed to produce cytokines when stimulated with anti-CD3 and anti-CD28 antibodies. Upon examining the cell population in BM, we did not detect any infiltration of T cells. In addition, we observed an increase in long term HSCs (LT-HSCs) and a decrease in short term HSCs (ST-HSC). We also observed the same phenomena in WT recipients of IFN-γ ARE KO BM. The results of this study suggest AA occurs when IFN-γ inhibits the generation of ST-HSCs from LT-HSCs, as opposed to infiltration of autoreactive T cells.

Molecular and Cell Biology, Virology, and Bioinformatics I

Oral Presentation

Tempol as a potential protective agent of nucleoside reverse transcriptase inhibitor (NRTI)-induced mitochondrial toxicity

Alexander T. Gibbons[1], Yongmin Liu[1], Phuonggiang Nguyen[1], James B. Mitchell[2] and Miriam C. Poirier[1]

[1]Carcinogen-DNA Interactions Section, LCBG; [2]Tumor Biology Section, RBB2, NCI, NIH, Bethesda, MD

NRTIs are essential components of the successful antiretroviral combination therapies used for treatment of HIV-1. However, during long-term therapy NRTIs may damage heart mitochondria, thus limiting clinical use of these drugs. Consequently, reducing NRTI-induced mitochondrial toxicity may benefit HIV-1-infected patients. Using H9c2 rat cardiomyocyte cultures exposed long-term to Zidovudine (AZT) or AZT plus Didanosine (ddI) we demonstrated NRTI-induced mitochondrial compromise. In an attempt to protect mitochondria from this damage we have used the stable free radical Tempol and its metabolite Tempol-H, cyclic nitroxides with antioxidant properties. H9c2 cells were exposed to 50 M AZT plus 50 M ddI for 15 passages (P15) in the presence and absence of 150 M Tempol or 150 M Tempol-H. The AZT/ddI combination caused moderate growth inhibition (< 30 %), and co-exposure with Tempol or Tempol-H did not restore cell survival. Mitochondrial oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using the Seahorse XF24 analyzer. As seen previously, AZT/ddI reduced maximal FCCP-uncoupled OCR. However co-exposure with Tempol and Tempol-H stimulated OCR, restoring the AZT/ddI-reduced uncoupled OCR by 22-91 %. Similarly, the uncoupled ECAR levels were increased by 15-46 % with Tempol or Tempol-H. Electron microscopic analysis also supported Tempols protective effects of AZT/ddI-induced mitochondrial morphological damage. Preliminary Western blot findings showed that Tempol and Tempol-H enhanced the expression of uncoupling protein-2 (UCP-2). Therefore Tempol and Tempol-H may protect cardiomyocytes from mitochondrial compromise induced by the NRTI combination AZT/ddI, and UCP-2 may play a role through mild uncoupling. We are currently evaluating potential mechanisms by which these compounds may act as mitochondrial protective agents.

Oral Presentation

Oxidative stress upregulates the latency-associated nuclear antigen protein of Kaposis Sarcoma-Associated Herpesvirus in chronically infected cells

Nicole E. Naiman, Duosha Hu, Katharine S. Davidoff, David A. Davis, Robert Yarchoan

HIV and AIDS Malignancy Branch, CCR, NCI

Kaposis sarcoma-associated herpesvirus (KSHV) is the causative agent for three HIV-associated malignancies: Kaposis sarcoma, primary effusion lymphoma, and multicentric Castlemans disease. Like other viruses, KSHV survives in cells by thwarting cellular mechanisms designed to activate apoptosis through oxidative stress or other means. We investigated the mechanisms by which KSHV may overcome oxidative stress induced by infection of B-cells. H2O2 was used to induce oxidative stress in KSHV-infected cells, and killed the cells in a dose-dependent manner. A pan-caspase inhibitor inhibited this cell death, indicating oxidative stress activated apoptosis. At the same time, H2O2 increased the levels of KSHV latency-associated nuclear antigen (LANA). LANA is the main latency-associated protein of KSHV and, among other functions, tethers the KSHV episome to cellular DNA. The increase in LANA due to oxidative stress was detected by western blot and immunofluorescence. The upregulation of LANA was also observed at the RNA level by quantitative PCR and by Northern blot. Interestingly, sequence analysis of the promoter region of LANA revealed three potential antioxidant response elements indicating that oxidative stress may directly upregulate LANA. While oxidative stress upregulated production of LANA, high levels of oxidative stress also altered the banding profile of LANA as determined by western blot. The alteration in the LANA profile also occurred in the presence of cyclohexamide, a protein synthesis inhibitor, indicating the change occurred post-translationally. Upon treatment with H2O2 in the presence of a pan-caspase inhibitor, the change in the LANA profile was inhibited, suggesting caspase activation results in cleavage of LANA. In support of direct cleavage by caspases, we identified a potential caspase cleavage site within the LANA sequence. We hypothesize that, in addition to maintaining the viral episome, LANA also functions to prevent the cell from undergoing apoptosis caused by oxidative stress.

Oral Presentation

Altering the Immunodominance Hierarchy of p55gag by DNA Vaccine Expressing Conserved Regions

George N. Pavlakis[1], Viraj Kulkarni[2], Antonio Valentin[1], Margherita Rosati[1], Niranjan Y. Sardesai[3], Beatriz Mothe[4], Christian Brander[4], Sylvie LeGall, David B. Weiner[5], Morgane Rolland[6], James I. Mullins[7], and Barbara K. Felber[2]

[1]Human Retrovirus Section; [2]Human Retrovirus Pathogenesis Section, VB, CCR, NCI-Frederick; [3]Inovio Pharmaceuticals Inc, Blue Bell; [4]IrsiCaixa-HIVACAT, Barcelona; Ragon Institute, Boston; [6]University of Pennsylvania, Philadelphia; [7]Military HIV Research Program, Rockville, [8]University of Washington, Seattle

Background: We developed strategies addressing two hurdles in the development of effective HIV vaccines: immunodominance and sequence diversity. Immunodominance may obscure/prevent reactivity against potentially protective epitopes, whereas responses to subdominant epitopes were associated with better virologic control. We tested the hypothesis that inclusion of conserved elements (CE) of the HIV proteome into a vaccine candidate, while excluding the variable regions, would help overcome problems of viral sequence diversity and potential negative effects of immunodominance. Methods: Two HIV p24gag CE plasmids were engineered to express 7 strictly conserved elements of 12-24 AA, arranged in collinear fashion covering 140 AA and representing 54% of p24gag. Core1 and Core2 differed by one toggle site per CE and provided potential epitopes found in >99% of all known HIV-1 M group sequences. These plasmids were used to immunize mice and macaques by IM injection followed by in vivo electroporation. Results: Core1 and Core2 elicited strong, cross-clade cellular and humoral responses that were similar in magnitude to those induced by p55gag in mice and macaques. Mapping studies showed that one or more CE were immunogenic. In contrast, macaques vaccinated with p55gag DNA vaccine did not develop immune responses to the CE, despite developing good responses to other regions in Gag. However, CE DNA primed macaques, upon subsequent boosting with a plasmid expressing p55gag, showed up to 10-fold increase of individual CE responses. This data demonstrate that responses to conserved regions recognize the complete Gag and, thus the infecting virus. Conclusions: A prototype p24gag CE vaccine induced strong and cross-clade cellular and humoral immune responses to different CEs. Vaccination with the p24gag CE DNAs altered the immunodominance hierarchy of Gag and focused the responses to conserved regions. Translation of this concept into clinical trials may provide a significant improvement in eliciting cross-clade cellular immune responses.

Oral Presentation

Cell cycle-dependent phosphorylation regulates BAG3 binding to Hsp70

Andrea D. Fischione, Ryan K. Henning, Mathew G. Angelos, Elise C. Kohn and Andrea K. McCollum

Molecular Signaling Section, Medical Oncology Branch, NCI, Bethesda, MD

BAG3 is a BAG domain-containing protein that binds the ATPase domai of Hsp70 and prevents release of Hsp70 client proteins. Recent reports indicate that knockdown of BAG3 with siRNA causes a disruption of cell cycle distribution at S and G2 phases. However, mechanisms underlying BAG3-mediated regulation of cell cycle progression remain unidentified. We found that BAG3 from cells synchronized in G2/M with nocodazole treatment migrated slower in an SDS-PAGE gel than BAG3 from asynchronous cells, or cells synchronized in S phase by a double thymidine block. We hypothesized that this mobility shift was due to BAG3 phosphorylation during G2/M. Incubation with calf intestinal alkaline phosphatase increased the mobility of BAG3 in nocodazole-treated cells, indicating that BAG3 is phosphorylated differentially during the phases of the cell cycle. BAG3 contains five potential sites for phosphorylation by Cdc2, a serine/threonine kinase responsible for mitotic progression. Knockdown of Cdc2 prevented the mobility shift that indicates phosphorylation of BAG3. Co-immunoprecipitation demonstrated that Cdc2 binds to BAG3 after nocodazole treatment but not in asynchronous cells. Additionally, Cdc2 was pulled down with a GST fusion protein containing the BAG domain of BAG3, but not GST control. Therefore, we conclude that Cdc2 binds the BAG domain of BAG3 and participates in the phosphorylation of BAG3 at the G2/M phase of the cell cycle. We stimulated BAG3 phosphorylation by treating with okadaic acid, an inhibitor of protein phosphatase activity. Treatment with either okadaic acid or nocodazole caused an inhibition of BAG3 binding to Hsp70, suggesting that phosphorylation regulates BAG3 binding to Hsp70. Immunoblot analysis of HeLa, Hey A8 and CCD-27Sk cells revealed that BAG3 expression is increased in S phase and decreased in G2/M phases compared to G1. These data suggest that phosphorylation may prevent BAG3 binding to Hsp70 and may target BAG3 for degradation during the G2/M phases.

Oral Presentation

SIVmac239 DNA and Virus Particle Vaccination Confers Protection From Infection and Disease Progression Upon Challenge With Heterologous SIVsmE660

Rashmi Jalah[1], Vainav Patel[2], Viraj Kulkarni[1], Antonio Valentin[2], Candido Alicea[1], Margherita Rosati[2], Agneta von Gegerfelt[2], Niranjan Y. Sardesai[3], Julian Bess, Jr.[4], Jeffrey D. Lifson[4], Brandon Keele[4], Ramara R. Amara[5], Harriett L. Robinson[6], Vanessa M. Hirsch[7], Yongjun Guan[8], David Venzon[9], David C. Montefiori[10], George N. Pavlakis[2], and Barbara K. Felber[1]

[1]Human Retrovirus Pathogenesis Section, [2]Human Retrovirus Section, Vaccine Branch, CCR, NCI-Frederick; [3]Inovio Pharmaceuticals, Inc., Blue Bell; [4]AIDS and Cancer Virus Program, SAIC, NCI-Frederick; [5]Yerkes National Primate Center, Atlanta; [6]GeoVax. Inc., Smyrna; [7]Viral Pathogenesis and Vaccine Section, NIAID, Bethesda; [8]Institute of Human Virology, Baltimore; [9]Biostatics and Data Management Section, CCR, NCI; Duke University Medical Center, Durham

Background: Macaques vaccinated with DNA vectors expressing SIVmac239 antigens develop potent immune responses able to reduce viremia upon high dose SIVmac251 challenge. To further improve immune responses and protection, protocols combining DNA with protein immunization (in the form of AT-2-inactivated viral particles) were tested. Methods: Three groups of Indian rhesus macaques (N=8) were vaccinated via IM electroporation (weeks 0, 8, 16 and 36): (a) 4x SIVmac239 DNAs only, (b) 4x coimmunization of DNAs with protein (AT-2-inactivated particles) and (c) 2 DNA vaccinations followed by 2 protein boosts. At 7 months after the last vaccination, the animals were challenged weekly with low dose heterologous SIVsmE660. Results: All vaccinees developed high and persistent humoral responses to SIV Gag and Env and the ability to cross-neutralize the heterologous SIVsmE660 Env. SIV-specific cellular responses were readily measured in blood and mucosal sites and remained high during the entire follow-up. Vaccinated macaques showed slower SIVsmE660 acquisition compared to nave controls (p=0.05; stratified for Trim5a genotype). Two of DNA-protein immunized macaques showed sterilizing immunity after 14 exposures, while all controls were infected by 6 exposures. These macaques had high and persistent Ab responses. Vaccinees had significantly lower peak viral loads (1.7 log, p=0.03) and 75% of vaccinees suppressed virus replication rapidly to undetectable levels (p= 0.026), and maintained normal CD4 counts (40 weeks of follow-up). Protection from infection was correlated with binding antibody to both SIVsmE660 and SIVmac251 at the day of challenge. Control of viremia correlated with the presence of vaccine-induced cellular immune responses (CD4+ Memory T-cells and IFNgamma+ Granzyme-B+ cytotoxic T-cells). Conclusion: DNA and protein based vaccine protocols are able to achieve rapid, high, persistent, broad and effective immune responses, which are able to prevent heterologous SIV infection at the portal of entry. These studies may significantly contribute to the development of an efficacious HIV/AIDS vaccine.

#10

Sequence specific effects on DNA and cell damage with the PARP inhibitor olaparib (AZD2281) and carboplatin

Mario Cedillo[1], John Hays[2], Jung-Min Lee[3], Matthew Angelos[4], and Elise Kohn[5]

Medical Oncology Branch, NCI, NIH

Background: We have found clinical activity of carboplatin (C) with the PARP inhibitor, olaparib (O), in BRCA1/2mut or BRCA-like breast and ovarian cancers. Current clinical trials are testing the hypothesis that PARP inhibition will sensitize tumors to platinums. We have examined sequence specificity of C and O combinations in cell lines, including two BRCA1mut (HCC1937, UWB1.289) and two BRCA-WT lines (OVCAR8, HeyA8), on the development of cell injury, DNA damage and DNA repair. Methods: Cell injury was measured with XTT assays. DNA damage was examined using Comet assay with tail quantification. DNA double strand break and double strand break repair were measured by counting gH2AX and RAD51 foci respectively using immunofluorescence. Platinum DNA-adduct formation was measured using atomic absorption spectrometry and normalized to DNA input. Results: Exposure to O prior to C for 24 hours (O>C) reduced the efficiency of double stranded DNA damage and increased DNA repair activity when compared to C alone or O+C measured by gH2AX and RAD51 foci/nuclei in BRCA1mut cell lines. Comet assay demonstrated decreased DNA damage in BRCA1mut cells treated with O>C compared to C and O+C. Cell cytotoxicity by XTT assay indicated O>C had decreased cytotoxic effects compared to C or O+C, seen in BRCA1mut cell lines. Platinum adduct formation was not affected by treatment with O in either BRCA1mut cell lines or BRCA-WT cell lines. Conclusions: The sequencing of O with C yields changes in cytotoxicity, DNA damage and DNA repair while platinum adducts were not altered by O exposure. This suggests pretreatment of BRCA-deficient cell lines with PARP inhibition may up-regulate other DNA repair pathways and lead to paradoxically less DNA damage than seen with C alone or C+O concurrently. Further studies are ongoing to evaluate this question.

#11

Lack of Top1mt impairs mtDNA metabolism

Ilaria Dalla Rosa[1], Hongliang Zhang[1], Takehiro Yasukawa[2], and Yves Pommier[1]

[1]Laboratory of Molecular Pharmacology, NCI, NIH, Bethesda, [2]Wolfson Institute for Biomedical Research, University College London, UK

Mitochondria contain a separate multi copy circular genome (mtDNA) that encodes essential components of the electron respiratory chain. Mitochondrial topoisomerase I (Top1mt) is exclusively targeted to mitochondria and its activity is required to overcome topological problems arising during mtDNA transcription and replication. Although conserved in all vertebrate, Top1mt is not essential for mouse development and its specific functions are currently under investigation. Here, we set out to study the biological roles of Top1mt in mitochondria. Using Top1mt knockout models, we show that lack of Top1mt alters mtDNA homeostasis: Top1mt deficient MEFs show increased mtDNA transcription rate and accumulate highly negatively supercoiled mtDNA. Moreover, Top1mt depletion impairs mitochondrial translation and leads to the production of a deficient respiratory chain. Taken together our results suggest a role for Top1mt in regulating mtDNA metabolism

#12

Coupling of Poly(ADP-ribose) polymerase (PARP1) and Tyrosyl-DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I (Top1)-mediated DNA damage

Benu Brata Das[1], Shar-yin N. Huang, Junko Murai and Yves Pommier

Laboratory of Molecular Pharmacology, CCR, NCI, NIH, Bethesda MD

PARP1 catalyze the covalent attachment of poly (ADP-ribose) chains (PAR) to a variety of proteins including themselves and chromatin associated proteins. Negatively charged PAR polymers facilitate the recruitment of repair factors such as XRCC1 and cause PARP to dissociate from the DNA. PARP inhibitors are effective antitumor agents in clinical development. They trap PARP on damaged DNA and enhance the activity of Top1 inhibitors. How PARP1 facilitates the repair of Top1-induced damage is not fully understood. One of the key repair enzymes for Top1 is Tdp1. Tdp1 hydrolyzes phosphodiester bonds at a DNA 3'-end linked to a tyrosyl moiety. This type of linkage is found in stalled Top1cc, and Tdp1 is implicated in the repair of such complexes. Here we show that PARP1 is activated in response to the Top1 poison camptothecin (CPT), with subsequent increase in PAR and Tdp1 levels in human cancer cells. Using pull-down experiments, we demonstrate that PARP1 directly interacts with Tdp1, enhances the catalytic activity of Tdp1, and poly(ADP-ribosylates) Tdp1. ADP-ribosylation increases the half-life of Tdp1 after DNA damage, and induces the formation of repair complexes including XRCC1 and Ligase III. The PARP inhibitor, veliparib (ABT-888) abrogates the formation of repair complexes between XRCC1 and Tdp1 and the formation of CPT-induced XRCC1 nuclear foci. Consistent with the PARP inhibitor experiments, PARP1 knockdown cells are deficient in forming Tdp1-XRCC1 complexes and XRCC1 foci in response to CPT. Increased DNA damage with PARP inhibitors is also evidenced from the rapid induction of the CPT-induced histone γH2AX, ATM activation and phosphorylation of Tdp1 at serine 81. Finally, by generating Tdp1 and PARP1 double knockout chicken DT40 cells we show PARP1 is epistatic with TDP1 for the repair of CPT-induced Top1cc. This work identifies a new mechanism by which PARP1 regulates Tdp1 function in cells and provides a rationale for combining PARP inhibitor with Top1 inhibitor in cancer.

#13

RNA Polymerase II Mutants of RPB1 Important for Transcription Fidelity in Saccharomyces cerevisiae Isolated Using a Novel Assay

Ashley S. Denney[1] **and Jeffrey N. Strathern**[1]

[1]Gene Regulation and Chromosome Biology Laboratory, CCR, NCI-Frederick

Transcription fidelity in vivo has long proved difficult to investigate since transcription errors are transient unlike permanent DNA events. Here we demonstrate the results of a novel assay whereby a transient transcription error is converted to a permanent genetic event and color phenotype in yeast colonies. We have designed a reporter gene as a target for Cre recombinase such that Cre restores the function of ADE6 and allows cells to produce a red pigment. In wild type colonies, an out-of-frame cre driven by a weak HIS3 promoter results in mostly white colonies with rare red sectors that result from rare Cre production. Transcription slippage over a homopolymeric track of adenines upstream of the out-of-frame cre has the potential to correct its reading frame so that functional Cre is produced. Thus it is possible to screen for mutants with elevated transcription slippage as evidenced by increased red sectoring in colonies. Previously we have shown that this assay is sensitive to an adenine-8-extra-base-one (A8EB1) track and demonstrates significant phenotypes with known slippage-prone RNA polymerase II mutants of the largest subunit, Rpb1, relative to wild type. Here we describe the isolation of numerous rpb1 mutations important for transcription slippage. These mutations localize largely to the secondary pore structure of Rpb1 although it is not yet known how they mediate transcription slippage. We are currently characterizing these mutants using lacZ quantification of transcription slippage and sensitivity to 6-Azauracil. Transcription fidelity is a vital cell process yet the consequences of transcriptional infidelity remain to be fully understood. Ultimately, we hope to contribute to this understanding as it relates to genomic stability, mutagenesis, and proteostasis, important factors underlying cancer development.

#14

A novel human monoclonal antibody disturbs Wnt signaling and inhibits hepatocellular carcinoma growth by targeting the heparan sulfate functional domain on Glypican3

Wei Gao and Mitchell Ho

Antibody Therapy Section, Laboratory of Molecular Biology, CCR, NCI-Frederick

Hepatocellular carcinoma (HCC) is the third most malignancy in the world. There is no effective treatment for HCC. Glypican-3(GPC3) is a proteoglycans highly expressed in HCC. Recent studies showed that GPC3 promotes HCC proliferation, which suggested that it might be a novel therapeutic target for liver cancer. Here we found that GPC3 was involved in HCC migration via the heparan sulfate chains of GPC3. Based on this, we isolated HS20, a new human monoclonal antibody against GPC3, by phage display technology. HS20 recognized the heparan sulfate chains of GPC3 and inhibited HCC cell migration by disturbing the interaction between GPC3 and Wnt3a, which consequently lead to the degradation of β-catenin. Many downstream genes of Wnt signaling like Fibronectin, E-cadherin, and VEGF were also down regulated upon the treatment of HS20. HS20 showed significant inhibition on tumor growth of HCC xenograft in mice. This is the first study to identify a human antibody against the HS domain on glypican and it will be beneficial for novel liver cancer therapy.

#15

A critical role for the C/EBPγ transcription factor in regulating in vitro and in vivo oxidative homeostasis

Christopher Huggins[1], Radek Malik[2], Diana Copeland Haines[3], and Peter Johnson[1]

[1]Laboratory of Cancer Prevention, CCR, NCI-Frederick; [2]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic; [3]Pathology/Histotechnology Laboratory, SAIC, NCI-Frederick

C/EBPγ is a member of the CCAAT/enhancer binding protein family of bZIP transcription factors. Despite being nearly ubiquitously expressed, its regulatory functions are not well understood. C/EBPγ heterodimerizes with other C/EBP proteins, and C/EBPβ:C/EBPγ dimers are the predominant C/EBP species in most cells. Development of Cebpg–/– embryos is grossly normal on a C57Bl/6 background, but the newborn pups do not survive past 48 hours. We demonstrate here that the most likely cause of death of mutant pups is impaired lung inflation. We also report that Cebpg–/– MEFs have severe proliferation defects, display delayed S-phase entry, and show elevated levels of senescence-associated β-Galactosidase. Gene expression profiling and pathway analysis identified increased expression of several genes involved in oxidative stress responses in mutant MEFs, suggesting deregulated oxidative homeostasis in these cells. Consistent with this idea, Cebpg–/– MEFs showed a four-fold increase in reactive oxygen species (ROS). Strikingly, N-acetyl-cysteine (NAC), a free radical scavenger and cysteine supplement, fully rescued the severe proliferation defect. Cysteine is a precursor in the synthesis of the critical cellular antioxidant, glutathione (GSH). Accordingly, we observed decreased GSH/GSSG levels in Cebpg–/– MEFs, providing a plausible explanation for elevated ROS. Furthermore, Cystathionine gamma-lyase (Cth), a gene encoding an enzyme involved in intracellular synthesis of cysteine, was reduced nearly 10-fold in Cebpg–/– MEFs. To determine whether the perinatal lethal phenotype is related to oxidative stress, we supplemented the food and water of mothers with NAC throughout pregnancy. This protocol led to significantly increased survival of Cebpg–/– pups, indicating that elevated ROS levels negatively influence the viability of developing mutant embryos/pups. In summary, loss of C/EBPγ results in increased oxidative stress, most likely due to a reduction in intracellular cysteine leading to impaired GSH synthesis.

#16

Distinct Regulatory Mechanisms and Functions for p53-Activated and p53-Repressed DNA Damage Response Genes in Embryonic Stem Cells

Mangmang Li[1], Yunlong He[1], Wendy Dubois[1], Xiaolin Wu[2], Jianxin Shi[3], and Jing Huang[1]

[1]Cancer and Stem Cell Epigenetics, LCBG, CCR, NCI; [2]Laboratory of Molecular Technology, SAIC-Frederick, Inc., NCI-Frederick; [3]Biostatistics Branch, Division of Cancer Epidemiology and Genetics, NCI

The tumor suppressor p53 is the guardian of the genome. Its roles in somatic cells have been well characterized in over past 30 years. Accumulating evidence also suggests that p53 play critical roles in regulating the differentiation of stem cells. Embryonic stem cells (ESCs) are the source of all the cell types in mammalian body. It is expected that ESCs have exquisite mechanisms to protect their genomic integrity, since any mutation in ESCs can cause the genome disruption of whole cell lineage. However the roles of p53 in embryonic stem cells have not been fully explored. Here, I employ genome-wide approaches, such as ChIP-Seq and gene expression microarray, to investigate the p53 regulatory circuit in ESCs. My results reveal that the p53-activated and p53-repressed genes are regulatorily and functionally separable. p53 activates its targets mainly through binding to the promoter region, while repressing its targets via binding to the distal region. Interestingly, I identify the interference with distal enhancer as a novel mode for p53-mediated repression. Further functional analyses demonstrate that p53-activated genes are linked to differentiation while p53-repressed genes are highly associated with ES/induced pluripotent stem (iPS) cell status. Importantly, the repressed genes include many master regulators of ESCs, such as Oct4, Nanog, Sox2 and n-Myc. Therefore, my results suggest that p53 protects the ESCs genomic integrity by inducing differentiation associated genes while repressing ESCs associated genes. p53 pathway is a major barrier to somatic cell reprogramming. However, the exact mechanism is yet clear. My study suggests that p53 may inhibit the reprogramming by repressing the expression of ESC master regulators. Many poorly differentiated tumors bear an ESC gene signature and p53 mutations. My future studies will concentrate on the differentiation regulation of ESCs by p53 mutants. Results from these studies could develop novel therapeutic strategies to treat poorly differentiated tumors.

#17

β-hairpin peptides induce pore formation in negatively charged model membrane system

Kshitij Gupta[1], Kevin Harlen[2], Ulrich Baxa[3], Anu Puri[1], Joel Schneider[2], and Robert Blumenthal[1]

[1]Membrane Structure and Function Section, Nanobiology Program, [2]Peptide Design and Materials Section, Chemical Biology Laboratory, CCR, [3]Electron Microscopy Laboratory, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD

Peptides with different structural conformations have been investigated to understand their mechanism of biological membranes perturbation using liposomes as a model membrane system. In the present investigation, we are exploring the membrane destabilizing properties of novel amphiphilic positively charged β-hairpin peptides, MAX1 and MAX35. For these studies we used small unilamellar liposomes (about 100 nm in diameter) consisting of a neutral phospholipid (POPC) and varying amounts of negatively charged phospholipid (POPS). MAX1 and MAX35 fold into β-hairpin conformations in the presence of negatively charged liposomes as shown by circular dichroism measurements and promote release of encapsulated solute Tb/DPA from those liposomes. The release was rapid and its extent depended on peptide to lipid and PS/PC ratios reaching 100% at a peptide/lipid ratio of about 1/6 in PS:PC 1:1 liposomes. A control peptide, MAX8V16E, did not fold and failed to promote solute release under identical conditions. Inclusion into the liposome of PEG-PE, which prevents Ca2+ - induced aggregation, fusion and leakage of POPS liposomes, did not inhibit β-hairpin peptide-induced leakage. Electron microscopy and dynamic light scattering measurements indicate no significant size or shape changes upon interaction of β-hairpin peptides with PEG-PE POPC/POPS (1:1) liposomes. On the basis of our experiments we propose that β-hairpin MAX peptides induce pore formation in the negatively charged model membrane system.

#18

Role of intracellular loops 1 and 3 in folding and cell surface expression of P-glycoprotein (ABCB1)

Khyati Kapoor[1], Jaya Bhatnagar[1], Eduardo Chufan[1], and Suresh V. Ambudkar[1]

[1]Laboratory of Cell Biology, CCR, NCI, NIH, Bethesda, MD

P-glycoprotein (ABCB1) is an ATP-Binding Cassette transporter that transports variety of structurally diverse compounds including anticancer drugs out of the cell. The catalytic cycle of ATP hydrolysis involves conformational changes in the transporter, which are poorly understood. In the absence of any structural information on human P-gp, computational models in the nucleotide-bound conformation were developed based on structures of Sav1866 and MsbA transporters. In homology model of human P-gp based on these structures, the adenine group of ATP makes hydrogen bond with conserved D164 and D805 on intracellular loop (ICL) 1 and ICL 3, respectively which are located at the interface of NBD and TM domains. We investigated the role of D164/D805 by substituting these residues with Cys residue in a cys-less background. The D164C/D805C mutant P-gp was expressed in High-Five insect cells as well as in HeLa cells by using the baculovirus expression system. In the insect cell membranes, the expression of the mutant P-gp was comparable to that of wild-type protein and exhibited the same ATP-binding affinity but a lower Vmax for ATP hydrolysis. However, when the mutant protein was expressed in HeLa cells with the BacMam baculovirus system, we found that the D164C/D805C mutant was poorly expressed (~10-15% compared to cysless-wild-type) at the plasma membrane and thus fails to transport almost all the drug substrates. HeLa cells transduced with mutant D164C/D805C baculovirus, grown in the presence of a chemical chaperone such as Cyclosporine A for 16-18 hrs resulted in expression of mutant P-gp at the cell surface similar to wild-type protein with normal transport function. This illustrates the role of residues in ICL 1 and ICL 3 for proper folding of P-gp at the plasma membrane and specifically shows the importance of D164 and D805 in interdomain interactions required for proper folding and assembly of functional transporter.

Chemistry, Pharmacology, and Structural Biology

Oral Presentation

Fully synthetic virus-like nanoparticles targeting prostate cancer cells

Yuhong Chen[1], Sergey G. Tarasov[2], Vadim Gaponenko[3], O.M. Zack Howard[1], J.J. Oppenheim[1], Marzena Dyba[2], Sriram Subramaniam[4], and Nadya I. Tarasova[1]

[1]Cancer and Inflammation Program, NCI-Frederick; [2]Structural Biophysics Laboratory, NCI-Frederick; [3]Department of Biochemistry and Molecular Genetics, University of Illinois in Chicago, Chicago; [4]Laboratory of Cell Biology, NCI-Bethesda

Virus particles have evolved as highly effective delivery systems. They are able to deliver whole proteins and large nucleic acid molecules to certain types of cells. Genetically engineered virus-like particles (VLPs) have been explored as drug delivery and imaging agents. However, generation of fully synthetic VLPs has not been achieved. We have found that properly derivatized synthetic analogs of transmembrane domains of membrane proteins not only inhibit the function of the corresponding receptor, but can self-assemble in aqueous solutions into virus-like particles with high precision (PNAS 2011, 108 (24), 9798). The described particles have intrinsic biological activity of the peptide they are composed of and fuse with cell spontaneously. However, the fusion is non-selective. We have now developed synthetic VLPs that fuse with cells like real viruses, in receptor mediated manner. Prevention of non-selective fusion was achieved by derivatization of the self-assembling peptide with polyethylene glycol chains of defined length. Addition of ligands recognizing receptors over expressed on prostate tumor cells allowed for the particles that fuse selectively with receptor-positive cells. Particles targeting gastrin-releasing peptide (GRP) receptor, luteinizing hormone-releasing hormone (LHRH) receptor and prostate-specific membrane antigen (PSMA) are remarkably stable, more than 99 % homogeneous in size and undergo receptor-mediated fusion with tumor cells. Development of fully synthetic virus-like particles establishes a foundation for generation of delivery systems with investigator- defined size, physical, structural properties and targeting ability for a wide range of biomedical applications.

Oral Presentation

Synergistic TRAIL sensitizers from Barleria alluaudii and Diospyros maritima

Emily L. Whitson[1], Han Sun[2], Cheryl L. Thomas[1], Curtis J. Henrich[1,3], Thomas J. Sayers[3,4], James B. McMahon[1], Christian Griesinger[2], and Tawnya C. McKee[1]

[1]Molecular Targets Laboratory, Molecular Discovery Program, CCR, NCI-Frederick, Frederick, MD; [2]Department of NMR Based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, Gttingen, Germany; [3]SAIC-Frederick, Inc., Frederick, MD; [4]Laboratory of Experimental Immunology, Cancer and Inflammation Program, CCR, NCI-Frederick, Frederick, MD

Tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL/Apo2L) is a member of the tumor necrosis factor (TNF) family of apoptosis triggering proteins. TRAIL is particularly important because it selectively induces apoptosis in cancer cells, while showing little to no effect in normal cells. However, TRAIL resistance has been widely documented, and there is evidence to suggest that combination chemotherapy regimens may be more effective than traditional cytotoxic mono-chemotherapy. In this manner, TRAIL activity may be restored by sensitizing tumor cells with certain chemical agents. Therefore, a high-throughput screen was developed to identify compounds that could sensitize tumor cells to the killing effects of TRAIL. Extracts from Barleria alluaudii and Diospyros maritima showed promising activity in the initial screen and were further investigated. As a result of this study, two naphthoquinone epoxides, 2,3-epoxy-2,3-dihydrolapachol (1) and 2,3-epoxy-2,3-dihydro-8-hydroxylapachol (2), both not previously isolated from natural sources, and the known 2-methyl anthraquinone (3) were identified from B. alluaudii. Time-dependent density functional theory (TD-DFT) calculations of electronic circular dichroism (ECD) spectra were utilized to establish the absolute configuration of 1 and 2. Additionally, five known naphthoquinone derivatives, maritinone (4), elliptinone (5), plumbagin (6), (+)-cis-isoshinanolone (7), and ethylidene-6,6'-biplumbagin (8) were isolated from D. maritima. Compounds 1, 2, and 4-6 showed varying levels of synergy with TRAIL. Maritinone (4) and elliptinone (5) showed the highest synergistic effect, with more than a three-fold increase in activity observed with TRAIL than with compound alone. No previous investigations of B. alluaudii, endemic to Madagascar, have been reported in the chemical literature. Compounds 1 and 2 are members of the lapachol family of quinones. Beta-Lapachol was evaluated by the NCI in the clinic during the 1970s, but was later withdrawn due to high levels of toxicity. The closely related beta-lapachone is currently in Phase II clinical trials for advanced solid tumors.

Oral Presentation

3DQSAR studies of BCR-ABL kinase inhibitor, Tasigna (nilotinib) and its derivatives for identification of pharmacophore essential for interaction with ABC drug transporters and target kinases

Suneet Shukla[1], Amanda P Skoumbourdis[2], Abdul Kouanda[1], Latoya Silverton[1], Matt Boxer[2], Damien Duveau[2], Tanaji Talele[3], Craig J Thomas[2], and Suresh V Ambudkar[1]

[1]Laboratory of Cell Biology, CCR, NCI; [2]NIH Chemical Genomics Center, Rockville. MD; [3]St. Johns University, Queens, NY

Tasigna (nilotinib) is a novel tyrosine kinase inhibitor (TKI), which has been used to treat chronic phase chronic myeloid leukemia patients, who develop resistance due to emergence of point mutations within the BCR-ABL kinase domain. It is also shown to be transported by ATP binding-cassette (ABC) drug efflux transporters, ABCB1 (P-glycoprotein, Pgp) and ABCG2 (BCRP). The interaction of Tasigna with ABC drug transporters is clinically relevant as it not only hampers the efficacy of the other chemotherapeutic drugs but also results in lower bioavailability of the drugs. Therefore, one of our goals is to develop specific high affinity inhibitor s of BCR-ABL kinase activity that exhibit no or minimal interactions with ABC drug transporters. We describe here 3D pharmacophore modeling and quantitative structureactivity relationship (QSAR) studies on a series of Tasigna analogs to propose the spatial arrangement of chemical features that are essential for inhibitory activity of Tasigna for BCR-ABL kinase activity and Pgp or ABCG2. Twenty six derivatives of Tasigna were synthesized by either deleting the key pyridine or imidazole ring or by changing functional groups in Tasigna. A set of in vitro experiments, including kinase activity and FACS based transport assays were carried out to evaluate their potency to inhibit ABC drug transporters and the target kinases. Sixteen, fourteen and ten compounds were selected as a QSAR dataset, respectively, for generating PHASE v3.1 pharmacophore models of BCR-ABL kinase, ABCG2 and Pgp inhibitors. The IC50 values of these derivatives against Pgp, ABCG2 or BCR-ABL kinase were used to generate pharmacophore features required for optimal interactions with these targets. A seven point pharmacophore (AADDRRR) for BCR-ABL kinase inhibitory activity, six point pharmacophore (ADHRRR) for ABCG2 inhibitory activity and seven point pharmacophore (AADDRRR) for Pgp inhibitory activity has been generated. The derived models clearly demonstrate high predictive power for test sets of BCR-ABL, ABCG2 and Pgp inhibitors. Though all of the pharmacophore models developed in this study showed excellent predictive power, limited number of compounds warrants caution when using these models for quantitative predictions. In aggregate, these results suggest that it may be possible to use 3D QSAR approach to develop a potent BCR-ABL kinase inhibitor that does not interact with ABC drug transporters.

Oral Presentation

Understanding HIV-1 Integrase and strand transfer inhibitors to overcome clinical resistance

Mathieu Mtifiot[1], Barry Johnson[2], Steven Smith[2], Xue-Zhi Zhao[3], Stephen Hare[4], Christophe Marchand[1], Terrence Burke[3], Peter Cherepanov[4], Stephen Hughes[2], and Yves Pommier[1]

[1]Laboratory of Molecular Pharmacology, CCR, NCI, NIH, 37 Convent Drive, Bethesda, MD; [2]HIV Drug Resistance Program and [3]Chemical Biology Laboratory, Molecular Discovery Program, CCR, NCI-Frederick, NIH, Frederick, MD; [4]Division of Infectious Diseases, Imperial College London, London, UK

Raltegravir (RAL) is the first HIV-1 integrase (IN) inhibitor approved by the FDA. The use of RAL in experienced and more recently in nave patients has become part of mainstream AIDS therapy. However, resistance to RAL has emerged as a limitation, involving IN mutations in three genetic pathways: N155H, G140S/A-Q148H/R/K and Y143H/R/C. We systematically investigated how these mutations affect IN enzymatic activity and RAL-resistance. We demonstrated that the clinically relevant IN mutations are sufficient to account for the phenotype of RAL-resistant viruses. With our set of mutant enzymes and the corresponding mutant viruses (over 20 mutants), we are in a unique position to study the resistant profile of next generation drugs. We first assessed the resistance profile of two drugs that are likely to be approved this year, elvitegravir and dolutegravir. Our studies show that both molecules have selective advantages over RAL. Elvitegravir overcomes the Y143 pathway but remains sensitive to the two other pathways. Dolutegravir presents an improved resistance profile against all the mutants except the Q148 pathway, which still induces 6-fold resistance. In parallel, we designed novel compounds and performed structure-activity studies with wild-type and mutant IN, and solved the atomic structure of our new inhibitors in the PFV intasome by X-ray crystallography. We observed a correlation between the ability to inhibit mutant enzymes and the selectivity for the inhibition of strand-transfer compared to 3-processing. These findings suggest that dual inhibition of 3-processing and strand-transfer could be a surrogate to assess the resistance profile of a drug. Finally, we are using raltegravir as a probe to evaluate integrase-DNA binding affinity using modified oligonucleotide to better understand the determinants of this selectivity. The long-term goal of our studies is to elucidate the molecular integrase-drug-DNA interactions and to explore new ways to target IN for inhibiting HIV replication.

Oral Presentation

Development of a high-throughput SUMOylation assay using fluorescent peptide probes and identification of SUMOylation inhibitors

Yeong Sang Kim and John Schneekloth Jr

Chemical Biology Laboratory, CCR, NCI-Frederick

Posttranslational modification of proteins with SUMO (small ubiquitin-related modifier) plays an important role in many cellular processes, including transcriptional regulation, subcellular localization of proteins, DNA repair, and signal transduction. SUMOylation is a covalent protein modification mediated by a cascade of enzymes, namely, SUMO-activating enzyme (E1), SUMO-conjugating enzyme (E2) and any of several E3 ligases. It results in an isopeptide bond formation between C-terminal Gly residue of SUMO and epsilon-amino group of a Lys residue in the target protein. Given the important role of SUMOylation in cellular homeostasis as well as a number of disease states such as infectivity and cancer, we have initiated a program to identify small molecule inhibitors of this process. We have developed a novel high-throughput SUMOylation assay using fluorescent peptide probes with SUMO consensus sequences. The SUMOylation is easily visualized by in-gel fluorescence imaging using fluorescent probes. In addition the assay can be performed in a high-throughput manner in 384-well plates. SUMOyaltion can be quantified as well as studied kinetically using capillary electrophoresis. The assay is tolerant to DMSO (0-5%), pH (7-10) and temperature (room temperature-40 deg). Real-time kinetics showed that under optimized conditions, the SUMOylation reaction remains linear for at least 2 hours at room temperature. Moreover, we have screened a series of small molecules using our assay and are in the process of characterizing hits.

#19

Molecular Characterization of a Common Drug-Binding Pocket of the Multidrug Resistance-linked P-glycoprotein

Eduardo Chufan, Khyati Kapoor, Hong-May Sim, Stewart Durell, and Suresh Ambudkar
Laboratory of Cell Biology, CCR, NCI

Multidrug resistance (MDR) is a clinical phenomenon in which cancer cells develop resistance to chemically diverse drugs. One of the majors players in MDR is P-glycoprotein (P-gp, ABCB1), which is a member of the ATP-binding cassette (ABC) transporter superfamily; this membrane protein utilizes energy from ATP hydrolysis for the efflux of a variety of chemically dissimilar compounds including anticancer drugs. P-gp is a typical ABC transporter with two transmembrane domains (TMDs) each containing six helices, and two nucleotide-binding domains (NBDs). In the TMDs, the transporter in apo conformation exhibits a large conical cavity opened to the cytoplasm in what appears to be a drug-binding pocket. Docking studies at this potential binding site were carried out for several substrates and modulators of P-gp such as cyclosporine A, tariquidar, valinomycin and 5-fluorosulfonylbenzoyl-5-adenosine. Residues interacting with the substrates/modulators in the docking studies were substituted with Cys in a Cys-less background. The mutants were expressed in High-Five insect cells as well as in bac-mam-baculovirus transduced HeLa cells for functional characterization. Biochemical studies provided evidence that substrates and modulators are not binding at their natural binding site in the P-gp mutants. However, ATP hydrolysis measurements show that substrates/modulators still interact with the proteins indicating that, upon mutation of the residues in the natural binding site, drugs bind at alternative sites. These observations suggest that the residues in the TMDs that are predicted to interact with drug substrates by docking studies are part of a common drug-binding pocket of P-gp; however, upon mutation drugs still bind at alternative sites suggesting that the substrate/modulator-binding pocket in P-gp is very flexible.

#20

Significant boost in potency by the bis-tetrahydrofuran (THF) and tris-THF moieties as P2 functional groups of HIV-1 protease inhibitors compared to that of THF against multi-drug-resistant HIV-1 protease

Ravikiran S. Yedidi[1], Kenji Maeda[1], David A. Davis[2], Debananda Das[1], Joshua D. Kaufman[3], Stephen J. Stahl[3], Paul T. Wingfield[3], David W. Smith[4], Venkateswararao Kalapala[5], Arun K. Ghosh[5] and Hiroaki Mitsuya[1, 6, *]
[1]Experimental Retrovirology Section and [2]Retroviral Disease Section, HIV and AIDS Malignancy Branch, CCR, NCI; [3]Protein Expression Laboratory, NIAMS, NIH; [4]Life Sciences Collaborative Access Team, Synchrotron Research Center, Northwestern University, Argonne, Illinois; [5]Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, Indiana; 6Kumamoto University Graduate School of Medical and Pharmaceutical Sciences, Kumamoto, Japan.

GRL-0519, a novel human immunodeficiency virus type-1 (HIV-1) protease inhibitor (PI) exhibits higher potency than darunavir (DRV), an FDA-approved second-generation PI against both wild-type and multi-drug-resistant HIV-1 strains. GRL-0519, DRV, and amprenavir (APV) consist of tris-THF (tetrahydrofuran), bis-THF, and THF moieties as P2 functional groups respectively. The current study focuses on the structure-function evaluation of GRL-0519, DRV and APV against a mutant HIV-1 protease isolated from a multi-drug-resistant clinical HIV-1 isolate (HIVMDR-A02), containing substitutions L10I, K45R, I54V, L63P, A71V, V82T, L90M, and I93L (PRMDR-A02). MTT-based assays were performed using human MT4 cells exposed to HIV-1NL4-3 or HIV-1MDR-A02 and cultured in the presence of GRL-0519, DRV, or APV. PRMDR-A02 was co-crystallized with GRL-0519, DRV, or APV. Crystal structures were solved using molecular replacement method and binding profiles were analyzed. GRL-0519 (IC50: 0.7 nM) was 3- and >300-fold more potent against HIV-1MDR-A02 compared to DRV and APV respectively. Crystal structures of PRMDR-A02 showed that the overall binding orientation of all three compounds did not change significantly compared to their corresponding wild-type HIV-1 protease crystal structures (PDB IDs: 3OK9, 2IEN, and 3EKV). All three compounds showed consistent direct polar contacts with the backbone amide nitrogen/carbonyl oxygen atoms of D29, D30, and D30 as well as with the bridging water molecule that connects to I50 and I50. The THF moiety of APV showed two contacts while the bis-THF and tris-THF moieties showed three contacts (two with D29 and one with D30) suggesting that both DRV and GRL-0519 have relatively a firmer grip on PRMDR-A02 in the S2 binding pocket compared to APV. GRL-0519 showed maximum contacts with PRMDR-A02 among the three compounds tested supporting its high antiviral potency against HIV-1MDR-A02. The bis-THF and tris-THF moieties significantly boost the potency of DRV and GRL-0519 respectively explaining their better inhibition profile compared to APV.

#21

Histone H4 K16Q mutation, an acetylation mimic, causes structural disorder of its N-terminal basic patch in the nucleosome

Bing-Rui Zhou[1], Hanqiao Feng[1], Rodolfo Girlando[2], Hidenori Kato[1], James Gruschus[3], Yawen Bai[1,*]

[1]Laboratory of Biochemistry and Molecular Biology, CCR, NCI, NIH, Bethesda, MD; [2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 3Laboratory of Molecular Biophysics, National Heart, Lung, and Blood Institute, Bethesda, MD

Histone tails and their post-translational modifications (PTM) play important roles in regulating the structure and dynamics of chromatin. For histone H4, the basic patch K16R17H18R19 in the N-terminal tail modulates chromatin compaction and nucleosome sliding catalyzed by ATP-dependent ISWI chromatin remodeling enzymes while acetylation of H4 K16 affects both functions. The structural basis for the effects of this acetylation is unknown. Here we investigated the conformation of histone tails in the nucleosome by solution NMR. We found that backbone amides of the N-terminal tails of histones H2A, H2B, and H3 are largely observable due to their conformational disorder. However, only residues 1-15 in H4 can be detected, indicating that residues 16-22 in the tails of both H4 histones fold onto the nucleosome core. Surprisingly, we found that K16Q mutation in H4, a mimic of K16 acetylation, leads to structural disorder of the basic patch. Thus, our study suggests that the folded structure of the H4 basic patch in the nucleosome is important for chromatin compaction and nucleosome remodeling by ISWI enzymes while K16 acetylation affects both functions by causing structural disorder of the basic patch.

#22

Crystal structures of the reverse transcriptase-associated ribonuclease H domain of xenotropic murine leukemia-virus related virus

Dongwen Zhou[1], Suhman Chung[2], Maria Miller[1], Stuart F.J. Le Grice[2] and Alexander Wlodawer[1]

[1]Protein Structure Section, Macromolecular Crystallography Laboratory, NCI-Frederick, Frederick, MD; [2]RT Biochemistry Section, Retroviral Replication Laboratory, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD

The ribonuclease H (RNase H) domain of retroviral reverse transcriptase (RT) plays a critical role in the life cycle by degrading the RNA strands of DNA/RNA hybrids. In addition, RNaseH activity is required to precisely remove the RNA primers from nascent (-) and (+) strand DNA. We report here three crystal structures of the RNase H domain of xenotropic murine leukemia virus-related virus (XMRV) RT, namely (i), the previously identified construct from which helix C was deleted, (ii), the intact domain, and (iii), the intact domain complexed with an active site α-hydroxytropolone inhibitor. Enzymatic assays showed that the intact RNase H domain retained catalytic activity, whereas the variant lacking helix C was only marginally active, corroborating the importance of this helix for enzymatic activity. Modeling of the enzyme-substrate complex elucidated the essential role of helix C in binding a DNA/RNA hybrid and its likely mode of recognition. The crystal structure of the RNase H domain complexed with β-thujaplicinol clearly showed that coordination by two divalent cations mediates recognition of the inhibitor.

#23

Potentiation of the novel Topoisomerase I inhibitor indenoisoquinoline LMP-400 by the cell checkpoint and Chk1-Chk2 inhibitor, AZD7762

Sheena M. Aris and Yves Pommier

Laboratory of Molecular Pharmacology, CCR, NCI, Bethesda, MD

Novel topoisomerase I (Top1) inhibitors are in clinical development to circumvent the drawbacks of camptothecins. Here we report molecular investigations into LMP-400, an indenoisoquinoline Top1 inhibitor in Phase 1 clinical trial by itself and in combination with the cell cycle checkpoint inhibitor, AZD7762. Drug effects on DNA replication and killing of cancer cells were examined. LMP-400 shows synergistic antiproliferative activity when combined with AZD7762 in human colon carcinoma HT-29 and HCT-15 cells. Inhibition of S-phase progression and bromodeoxyuridine incorporation were similarly induced by LMP-400 and camptothecin (CPT) and were abrogated by AZD7762. Replication studied by single DNA molecule analyses and immunofluorescence microscopy (molecular combing) showed rapid inhibition of fork progression in response to LMP-400 treatment with subsequent recapitulation after AZD7762 addition. AZD7762 inhibited both the activation/autophosphosphorylation of Chk1 and Chk2 at nanomolar concentrations in LMP-400-treated cells. This potent dual inhibition of Chk1 and Chk2 by AZD7762 was below the drug concentrations required to abrogate cell cycle inhibition and produce synergism with LMP-400. Also, the synergism was independent of Chk2 both in Chk2-complemented HCT-15 and Chk2 knockout HCT-116 cells, suggesting additional mechanisms for cell cycle abrogation by AZD7762. Our study demonstrates the rationale for combining cell cycle checkpoint inhibitors with the novel non-camptothecin indenoisoquinoline Top1 inhibitors.

#24

A Novel ERK2 Inhibitor is Superior to MEK1/2 Inhibitor AZD6244 in a Subset of Pancreatic Cancer Cell Lines

Anne Miermont[1], Holger Pflicke[1], Harshini Chinnasamy[1], Craig J. Thomas[2], Udo Rudloff[1]

[1]GI Oncology and HBP Section, Surgery Branch, CCR, NCI, and [2]NIH Chemical Genomics Center, National Human Genome Research Institute, NIH

Background: Intense efforts to improve current molecular therapy in pancreatic ductal adenocarcinoma (PDAC) focus on suppression of the Mitogen-Activated-Protein-Kinase (MAPK) pathway. Inhibitors of the MAPK kinase (MEK1/2), a downstream target of the frequently mutated RAS oncogene product have unfortunately not yielded their promising efficacy in phase II clinical trials in PDAC patients and currently do not surpass results of the largely ineffective 2nd and 3rd line chemotherapy options for this deadly disease. A novel Extracellular-signal-Regulated-Kinase-2 (ERK2) inhibitor, NCGC00242487-01, has been recently developed as a potent and selective compound. We hypothesized that inhibition of ERK2, downstream of MEK1/2, using NCGC00242487-01 is superior to inhibition of MEK1/2 by AZD6244. Methods: NCGC00242487-01 was compared to AZD6244 in 64 PDAC cell lines to evaluate Growth inhibition (GI) and Lethal Dose (LD) at 72hr with increasing concentrations of each compound. Apoptosis was quantitated by Annexin V/PI in a time-course experiment. Results: A subset (18.75%, 12/64) of NCGC00242487-01-treated PDAC lines showed GI50 concentrations of ≤1M. Moreover, LD50 of ≤10M with a left shift of the LD curve compared to AZD6244 LD curve or only an LD curve left shift without apparent cell death at ≤10M was observed with NCGC00242487-01 in 42.2% of the cell lines tested. In contrast, AZD6244 seemed to predominantly cause GI at 1M. Assessment of apoptosis by the AnnexinV/PI assay demonstrated that NCGC00242487-01 can induce increased rates of apoptosis, up to ~50% apoptosis, compared to AZD6244, starting in the sub-molar concentrations, in a subset of the PDAC lines. Conclusion: Our results suggest that this novel ERK2 inhibitor increased apoptosis compared to the MEK1/2 inhibitor AZD6244 in a subset of PDAC lines. This finding may lead to a more effective therapeutic strategy than MEK1/2 inhibition in some PDAC. Further development will require validation of ERK2-specific target inhibition and in-vivo efficacy studies in mouse models.

#25

Determination of conformational changes in the ATP sites during catalytic cycle of P-glycoprotein (ABCB1)

Hong-May Sim, Jaya Bhatnagar and Suresh Ambudkar

Laboratory of Cell Biology, CCR, NCI, NIH

P-glycoprotein (P-gp) is an ATP-binding cassette (ABC) efflux transporter implicated in multidrug resistance in cancer cells. The transport mechanism of P-gp is still not fully understood. The C431/C1074 residues in the Walker A motif of nucleotide-binding domains (NBDs) are highly conserved and these residues are excellent reporter sites to study the interaction between the two NBDs during the catalytic cycle. The chemical disulfide cross-linking of these residues in a cysless background can be observed in the presence of two cross-linkers, M14M and M17M with spacer arm length of 20 and 25 respectively. However, cross-linking with either M14M or M17M were prevented in the ADP-vanadate trapped (closed) conformation but not in the presence of ATPγS indicating that the distance or accessibility between residues 431 and 1074 changes upon ATP hydrolysis but not with ATP binding. In addition, the accessibility of C431, C1074 alone or together (C431/C1074) in both the apo and closed conformations of P-gp was determined using fluorescein-5-maleimide (FM), a thio-specific fluorescent probe. Different extent of labeling was observed among the cysteine mutants, with C1074 having 1.4-fold and 2-fold higher labeling by FM compared to C431 in the apo and closed conformations respectively, suggesting that accessibility of C1074 is greater than C431 in both conformations. In summary, NBDs of human P-gp are closer (20-25) compared to mouse P-gp and the cysteines in Walker A are differentially accessible in the apo and closed conformations.

#26

NF1-Dependent Tumors Suppressing Compounds from Zanthoxylum armatum

Krishna Devkota, James McMahon and John Beutler

Molecular Targets Laboratory, Molecular Discovery Program, NCI, Frederick, MD

The neurofibromatosis type 1 (NF1) based bioassay-guided phytochemical investigation on extracts and compounds from the widely used spice of the plant Zanthoxylum armatum collected from Nepal led to the isolation of four new and five known Sanshool compounds. The structures of all compounds were established by using modern spectroscopic techniques, including 1D- and 2D-NMR analysis and comparison with previously reported data. Most of the compounds possess significant NF1-dependent activity.

Genetics, Genomics, and Proteomics

Oral Presentation

miR487b is a Tumor Suppressor Silenced by Epigenetic Mechanisms during Tobacco-Induced Pulmonary Carcinogenesis

Sichuan Xi[1], Hong Xu[2], Jigui Shan[3], Yongguang Tao[2], Mary Zhang[1], Suzanne Inchauste[1], Leandro Mercedes[1], Julie A. Hong[1], David S. Schrump[1]

[1]Thoracic Oncology Section, Surgery Branch, CCR, NCI, Bethesda, MD; [2]Laboratory of Cancer Prevention, NCI, Frederick, MD; [3]Advanced Biomedical Computing Center, SAIC-Frederick, Inc., NCI, Frederick, MD

Limited information is available regarding the mechanisms by which miRNA alternations contribute to initiation and progression of lung cancer. In order to examine this issue, array techniques were used to assess miRNA expression profiles in normal small airway epithelial cells (SAEC) and immortalized human bronchial epithelial cells (HBEC), as well as Calu-6 and H841 lung cancer cells cultured in normal media (NM) with or without cigarette smoke condensate (CSC). Under relevant exposure conditions, CSC significantly decreased miR-487b expression in lung cancer cells and normal respiratory epithelia. miR-487b was significantly down-regulated (1.8-38.7 fold) in resected lung cancers relative to adjacent normal tissues. Software-guided analysis revealed numerous potential targets for miR-487b including Wnt5a, SUZ12, BMI1, c-MYC and K-ras. Constitutive over-expression of miR-487b inhibited, whereas depletion of endogenous miR-487b enhanced expression of Wnt5a, BMI1, SUZ12, c-MYC and K-ras in SAEC and Calu-6 cells. Repression of miR-487b coincided with increased recruitment of SUZ12 and BMI1 to Dkk-1, SFRP1, SFRP4, and WIF-1 promoter regions, and down-regulation of these genes in normal respiratory epithelia and lung cancer cells. RNA cross-link immunoprecipitation (CLIP) experiments confirmed direct interference of miR-487b with Wnt5a, BMI1, SUZ12, c-MYC, and K-ras transcripts. CSC increased CpG methylation and induced recruitment of SUZ12 and BMI1 to the miR-487b genomic locus. Deoxyazacytidine (DAC) induced miR-487b expression, and markedly attenuated CSC-mediated miR-487b repression. CSC-mediated repression of miR-487b coincided with de novo nucleosome occupancy in nucleosome free regions near miR-487b genomic sites, and decreased levels of H2AZ and TCF-1 within these regions. Epigenetic silencing of miR-487b by CSC was mediated by TGF-β1. Over-expression of miR-487b significantly decreased proliferation, invasion and tumorigenicity of lung cancer cells; knock-down of miR-487b increased growth and invasion of these cells. Collectively, these data suggest that epigenetic silencing of miR-487b promotes pulmonary carcinogenesis, in part, by activation of genes encoding proteins implicated in maintenance of cancer stem cells.

Oral Presentation

Risk alleles of four prostate cancer associated variants within the 8q24.2 region show increased interaction with androgen receptor

McAnthony Tarway, Wei Tang and Ludmila Prokunina-Olsson

Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD

Genome-wide association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) in the 8q24.2 chromosomal region as candidate risk factors for prostate cancer (PrCa). Normal prostate development as well as prostate tumor initiation and progression greatly depend on androgen receptor (AR) a testosterone-activated transcription factor which binds to androgen responsive elements (AREs) and triggers transcription of target genes. We hypothesized that some PrCa risk associated SNPs might affect AR binding sites. From the 600-Kb 8q24.2 region we selected 10 SNPs significantly associated with PrCa risk in 7 GWAS and consistently replicated in follow-up studies. We identified 269 proxy variants in high linkage disequilibrium (LD; r2>0.8) with these 10 SNPs based on the 1000 Genomes Project reference set. The same genomic region included 4 AR binding sites identified by genome-wide AR-chromatin immunoprecipitation (ChIP) studies both in LNCaP (androgen-dependent) and LNCaP-abl (androgen-independent) prostate cancer cell lines. All 4 of these AR binding sites overlapped with locations of PrCa GWAS SNPs or their proxies and shared a common feature the presence of a canonical ARE sequence and a binding site for the C/EBPα transcription factor. DNA-protein interaction studies confirmed binding of AR and C/EBPα to these variants. Our results suggest that interaction of PrCa-associated genetic variants with AR and C/EBPα can represent alternative mechanisms specifically important for development of hormone-refractory PrCa.

Oral Presentation

Functional exploration of CCNE1 splicing forms and link to bladder cancer

Indu Kohaar[1], Alexa Scott-Johnson[1], Yi-Ping Fu[1], Wei Tang[1], Liliana Guedez[2],Patricia Porter-Gill[1], Ludmila Prokunina-Olsson[1]

[1]Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD; [2]Radiation Oncology Branch, CCR, NIH, Bethesda, MD

A recent genome-wide association study (GWAS) identified a single nucleotide polymorphism (SNP), rs8102137, located 6 Kb upstream of the cyclin E1 gene (CCNE1) on chromosome 19q12, as a risk factor for bladder cancer (OR=1.13, p=1.7x10-11, Rothman et al, Nat Gen, 2010). CCNE1, which encodes a cell cycle protein, functions as a regulator of cyclin dependent kinases (CDK) and therefore is an important cancer susceptibility gene. mRNA expression analysis for bladder normal and tumor tissue samples showed higher expression for total CCNE1 mRNA in bladder tumors (n=42) compared to adjacent normal bladder tissue samples (n=41, 3.7 fold, p=2.7x10-12). By RNA-seq analysis in normal and tumor bladder tissue samples, we have identified two alternative splicing forms of CCNE1 of the potential importance for bladder cancer with deletions of exon 5 (CCNE1_5del) and exon 7 (CCNE1_7del) respectively. CCNE1_5del form lacks 49 aa within cyclin box, and is predicted to be non-functional due to its inability to form complex with cdk2. CCNE1_7del form has a deletion of 45 aa downstream of cyclin box and its functional significance remains unclear. Expression analysis with the splicing form-specific TaqMan assays validated the presence of these forms and showed correlation with several bladder cancer associated genetic variants in this region. Confocal microscopy analysis of recombinant CCNE1 isoforms also revealed that these variants are expressed in cytoplasm while wild type CCNE1 expresses in nucleus. Also, cell cycle analysis of transiently transfected HeLa cells with these expression constructs reveal that more cells were arrested in G0/G1 phase after transfection withalternative forms compared to transfected wild type CCNE1. To functionally characterize these protein isoforms ChIP-seq is being carried out to identify molecular targets of all CCNE1 isoforms. The results suggest that bladder cancer-associated genetic variants within the CCNE1 gene might contribute to altered cell cycle regulation by CCNE1 protein isoforms.

Oral Presentation

Identification and Functional Characterization of a Novel Splicing Form of the TPCN2 Gene on 11q13.3 Within a Region Associated with Prostate Cancer Risk

Nilabja Sikdar[1], Charles C. Chung[1], McAnthony Tarway[1], Wei Tang[1], Stefan Ambs[2], Ludmila Prokunina-Olsson[1], and Stephen J. Chanock[1]

[1]Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics; [2]Laboratory of Human Carcinogenesis, CCR, NCI, NIH, Bethesda, MD

Prostate cancer (PrCa) is the most frequently diagnosed cancer in males in developed countries. Genome-wide association studies (GWAS) for PrCa have discovered over 45 associated loci, including 3 independent signals on 11q13.3. Our resequencing and fine-mapping analysis nominated a single nucleotide polymorphism (SNP), rs10896438, located within a non-coding region, as the main association signal in this region. Rs10896438 resides between an EST DA379985 located 20 Kb telomeric and the TPCN2 gene located 92 Kb centromeric of this marker. We found expression of DA379985 in normal and tumor prostate as well as in other human tissues bladder, breast, cervix, ovary and testis. In 71 primary prostate tissue samples, DA379985 was expressed significantly lower in prostate cancer tissues compared to normal prostate tissue (p=0.034), however, we observed no association between rs10896438 and DA379985 mRNA expression. A rapid amplification of cDNA ends (RACE) experiment showed that this EST is a part of a novel splicing form of TPCN2 (TPCN2.1), encoding a protein of 520 aa instead of the TPCN2 protein of 752 aa. Confocal imaging of cells transiently transfected with expression constructs for TPCN2 and TPCN2.1 showed cytoplasmic lysosomal expression for both proteins. In conclusion, we found that the prostate cancer associated variant rs10896438 at 11q13.3 is located within a novel splicing form of TPCN2, expressed both in normal and tumor prostate tissue. Currently, we are exploring the possible functional role of TPCN2.1 in prostate cancer.

Oral Presentation

Identifying and Cloning the Hard Stuff from MCF7 breast cancer cell line: DNA Palindromes

Hui Yang[1], Alison Rattray[1], Natalia Volfovsky[2], Bob Stephens[2], Hisashi Tanaka[3], Claudia Stewart[4], Sandra Burkett[5], Xiongfong Chen[2], and Jeffrey Strathern[1]

[1]Gene Regulation and Chromosome Biology Laboratory, NCI-Frederick, Frederick, MD; [2]ABCC / ISP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [3]Department of Molecular Genetics, Cleveland Clinic, Cleveland, OH; [4]LMT/ATP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [5]Comparative Molecular Cytogenetics Core Facility, MCGP, NCI-Frederick, Frederick, MD

DNA palindromes are inverted DNA repeats that cause genome instability by spontaneously extruding into an intra-strand DNA cruciform structure. These alternate structures interfere with normal basic cellular processes such as replication, transcription and DNA repair. In addition to their instability, the formation of a DNA palindrome is a gene amplification process, hence it is no surprise that palindromes have been found non-randomly distributed in human cancer cells. So far no progress has been made to understand the mechanism of palindrome-associated gene amplification in cancers. The instability of long palindromes makes them hard to study. They cannot be cloned in E.coli, and they cannot be amplified by traditional PCR because intra-strand annealing results in a requirement for strand displacement in each PCR cycle. Taq polymerase is inefficient at strand displacement meaning that sequencing protocols (Illumina or 454) that are dependent on PCR amplification fail on DNA palindromes. We made progress in the identification, cloning, and sequencing of DNA palindromes. In addition, we developed systems to generate denovo palindromes. Here we report our analysis of DNA palindromes from MCF-7, a widely used cell line model for estrogen positive breast cancer. Chromosome rearrangement in MCF-7 has previously been studied by spectral karyotyping, comparative genomic hybridization (CGH), array CGH, single nucleotide polymorphism arrays and gene expression arrays. These studies are unable to detect palindromes due to the instability of palindrome in BAC clones and the inability to directly sequence palindromes. We employed a new strategy to identify palindromes by using DNA snap-back (GAPF) sequencing of MCF-7 genomic DNA. We have mapped the center of palindrome using Real-Time PCR and Inversion PCR. We have identified over 100 potential palindromes from MCF-7 using this strategy. And we successfully mapped and sequenced the spacers of four palindromes and cloned their junctions. The sequences of novel junctions indicate that NHEJ might be the mechanism of palindrome formation in MCF7. We also found that the locations of the four palindromes were close to the highly amplified regions on the chromosome, indicating that the palindrome formation might be an early event of gene amplification in cancer genome. Recently we have worked with the PacBio NextGen sequencing platform. It uses a DNA polymerase capable of strand displacement. We demonstrated that it is capable of sequencing DNA palindromes. We will apply our approach to study primary tumors and investigate the mechanism of palindrome-associated gene amplification in human tumors.

#27

Mutations in the ABCC6 Gene and Ten Documented Modifier Genes in Patients with PXE

Alexander Borsa[1], Sharon Terry[2], Michael Dean[1], and Karobi Moitra[1]

[1]Laboratory of Experimental Immunology, CCR, NCI; [2]PXE International Inc.

Pseudoxanthoma Elasticum (PXE) is an autosomal recessive disorder and is characterized by the mineralization of connective tissues in the body. PXE primarily manifests itself in the tissues of the skin, eyes, cardiovascular system, and gastrointestinal system, and is estimated to affect anywhere between 1 in 100,000 and 1 in 25,000 people. While there is no known treatment effective at combating PXE, it has been proposed that PXE is caused primarily by mutations in the ABCC6 gene. This gene encodes the transmembrane protein ABCC6, which functions as a transporter and is primarily expressed in the kidneys and liver. PXE is a disease with high phenotypic variability and over 100 documented mutations. We have analyzed pedigrees of over 26 families and retrieved DNA samples from individuals affected by PXE. We are in the process of sequencing the DNA of individual PXE patients from these families. We will use genetic sequence display programs such as Sequencher to analyze the genetic makeup of affected individuals for the presence of novel mutations of the ABCC6 gene, in addition to ten documented modifier genes. By identifying mutations in these genes, correlations may be made between an individuals genetic makeup and the specific manifestations of PXE in that individual. These correlations can further be used to identify the prognosis of individuals via genotyping, even if no prior symptoms of PXE have been manifested in the individual. In addition, new information on the genetics of PXE can be used to identify how amino acid variability affects the ABCC6 protein and, in turn, the disease itself.

#28

A cryptic BAP1 splice mutation in a family with uveal and cutaneous melanoma, and paraganglioma

Karin Wadt[1]*, Jiyeon Choi[2]*, Joon-Yong Chung[3], Jens Kiilgaard[4], Steffen Heegaard[4,5], Krzysztof T. Drzewiecki[6], Jeffrey M. Trent[7], Stephen M. Hewitt[3], Nicholas K. Hayward[8], Anne-Marie Gerdes[1], Kevin M. Brown[2,7]

[1]Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; [2]Laboratory of Translational Genomics, NCI, Bethesda, MD; [3]Laboratory of Pathology, CCR, NCI, Bethesda, MD; [4]Department of Ophthalmology, Glostrup University Hospital, Glostrup, Denmark; [5]Eye Pathology Section, Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark; [6]Department of Plastic Surgery, Breast Surgery and Burns Unit, Rigshospitalet, Copenhagen, Denmark; [7]Translational Genomics Research Institute, Phoenix, AZ; [8]Oncogenomics Laboratory, Queensland Institute of Medical Research, Brisbane, QLD, Australia
*These authors contributed equally to this manuscript

Background High penetrance BRCA1 associated protein-1 (BAP1) mutations have recently been reported in families with uveal or cutaneous malignant melanoma (UMM, CMM) as well as mesothelioma. To date, germline BAP1 mutations in these families have unambiguously resulted in protein truncation. Here we report a novel cryptic splice mutation of BAP1 in a Danish family with multiple UMM cases, as well as patients with a unique CMM phenotype, and paraganglioma. Methods One CMM and two UMM patients from a multiple-case UMM family were whole-exome sequenced. Predicted splice variant was analyzed in blood RNA using RT-PCR followed by Sanger sequencing. Loss of heterozygosity was examined on DNA isolated from cancer tissue. Results An apparent missense mutation of BAP1 was identified in UMM, CMM, paraganglioma, breast cancer, and suspected mesothelioma cases of the family. This mutation creates a strong cryptic splice donor, resulting in aberrant splicing and a truncating frameshift of the BAP1 transcript. Somatic loss of the wild-type allele was also confirmed in the UMM, and paraganglioma tumors. Conclusions Our findings further support BAP1 as a cancer susceptibility gene and extend the potential predisposition spectrum to paraganglioma. Further, these data highlight the need for rigorous bioinformatic assessment of apparently benign, novel coding mutations in larger whole-exome and genome sequencing studies.

#29

Allelic variant of mechanistic target of rapamycin (mTOR) induced differential expression of multiple miRNAs implicated in interactions with upstream and downstream targets of mTOR

Joy Gary[1,2], Shuling Zhang[1], Dena Tran[1], Aleksandra Michalowski[1], and Beverly Mock[1]

[1]Laboratory of Cancer Biology and Genetics, NCI, CCR, NIH; [2]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University

miRNAs are small, non-coding RNAs that regulate multiple genes involved in cell growth, development, and disease states by binding to the 3 untranslated region of the target mRNA and inhibiting translation or inducing degradation. The PI3K/AKT/mTOR pathway is frequently dysregulated in cancer and mechanistically often involves activation of growth factor receptor pathways, mutations, PTEN loss, and AKT amplification. Allelic variation and mutations in mTOR are relatively rare. Our lab discovered an allelic variant of mTOR (R628C; C1977T) in BALB/c mice, which predisposes the mice to development of pristane-induced plasma cell tumors (PNAS 100:14982). We recently developed a knock-in (KI) B6;129 mouse by homologous recombination that carries the BALB/c allele (C at nucleotide 1977) of mTOR (Blood 117:1228). Since miRs tend to control several genes simultaneously, we compared miR expression between wild-type (WT; mTORT1977) and mTORC1977 KI mice. B220+ splenic B-lymphocytes were isolated from WT and KI mice with and without pristane induction. miRNA was extracted from the splenocytes and a Nanostring miRNA expression assay was performed. BRB-ArrayTools was used to analyze differences in expression, and pathway analyses were performed with Ingenuity. In the Nanostring assay, several miRs were found to be differentially expressed. Those that were found to be significantly higher ($p < 0.05$) in the KI were miR30a (2-fold), miR101 (3-fold), and miR423-5p (1.5-fold). Similar trends were found with qRT-PCR. Pathway analysis in Ingenuity Pathway Analysis and evaluation of predicted targets in TargetScan revealed that miR30a likely interacts indirectly with AKT1 which is upstream of mTOR, and EIF4E, which is downstream. miR101 likely interacts with mTORC1. Finally, miR423-5p likely interacts indirectly with AKT1 and directly with 4EBP-1 (downstream of mTOR and hypophosphorylated in KI mice). Further exploration of the relationship of these miRs with the allelic variant of mTOR is needed to establish their role in the mTOR pathway.

#30

5HoxD-GLI3 antagonism regulates digit joint specification and phalanx number

Bau-Lin Huang, Aki Furusawa, and Susan Mackem

Cancer and Developmental Biology Laboratory, NCI-Frederick

During limb development, the number and positioning of joints is one of the main morphologic hallmarks of digit identity. The digit phenotypes in 5Hoxd and in Gli3 mutant mice suggest their role in controlling normal joint formation. However, the mechanism by which 5Hoxd genes and Gli3 contribute to digit identity in this manner at later stages is not understood. In the 5Hoxd mutant (Hoxd11-d13-/-), expression of Wnt ligands and other joint markers are reduced, and formation of early joint interzones is disrupted. We have used lineage analysis to show that joint precursors adopt a cartilage fate in 5Hoxd mutant embryos. Conversely, enforced 5Hoxd expression strongly inhibits chondrogenesis, both in vivo and in micromass cultures, reminiscent of canonical Wnt pathway activation, which plays a key role in joint formation. We have found that introducing a stabilized β-catenin allele restores normal joint formation, suggesting that 5Hoxd genes may act in a common pathway with β-catenin to regulate early joint cell fate. Normal joint formation is also rescued in 5Hoxd;Gli3 compound mutant embryos. Most strikingly, we have found that loss of only one copy of Hoxd11-d13 in Gli3 mutant embryos restores normal wild-type triphalangeal digit morphology. 5Hoxd proteins functionally antagonize Gli3R and our results suggest that the balance between 5Hoxd-Gli3 activity regulates both joint formation and normal phalangeal morphology. In ongoing work, we are analyzing changes in gene expression regulated by 5Hoxd-Gli3 balance that may underlie determination of joint precursor cell fate and normal phalanx morphogenesis.

#31

Mapping of the UGT1A locus identifies an uncommon coding variant that affects mRNA expression and protects from bladder cancer

Wei Tang[1], Yi-Ping Fu[1], Jonine D. Figueroa[2], Nria Malats[3], Montserrat Garcia-Closas[2,4], Nilanjan Chatterjee[2], Manolis Kogevinas[5-8], Dalsu Baris[2], Michael Thun[9], Jennifer L. Hall[10], Immaculata De Vivo[11], Demetrius Albanes[2], Patricia Porter-Gill[1], Mark P. Purdue[2], Laurie Burdett[12], Luyang Liu[1], Amy Hutchinson[12], Timothy Myers[12], Adonina Tardn[7,13], Consol Serra[14], Alfredo Carrato[15], Reina Garcia-Closas[16], Josep Lloreta[17], Alison Johnson[18], Molly Schwenn[19], Margaret R. Karagas[20], Alan Schned[21], Amanda Black[2], Eric J. Jacobs[9], W. Ryan Diver[9], Susan M. Gapstur[9], Jarmo Virtamo[22], David J. Hunter[23], Joseph F. Fraumeni Jr.[2], Stephen J. Chanock[1], Debra T. Silverman[2], Nathaniel Rothman[2], Ludmila Prokunina-Olsson[1]

[1]Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; [2]Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; [3]Spanish National Cancer Research Centre, Madrid, Spain; [4]Division of Genetics and Epidemiology, Institute of Cancer Research, London, UK; [5]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain; [6]Municipal Institute of Medical Research, Barcelona, Spain; [7]CIBER Epidemiologa y Salud Pblica (CIBERESP), Barcelona, Spain; [8]National School of Public Health, Athens, Greece; [9]Epidemiology Research Program, American Cancer Society, Atlanta, GA; [10]Lillehei Heart Institute, Department of Medicine, University of Minnesota, Minneapolis, MN; [11]Channing Laboratory, Department of Medicine, Brigham and Womens Hospital, Boston, MA; [12]Core Genotype Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [13]Universidad de Oviedo, Oviedo, Spain; [14]Universitat Pompeu Fabra, Barcelona, Spain; [15]Ramn y Cajal University Hospital, Madrid, Spain; [16]Unidad de Investigacin, Hospital Universitario de Canarias, La Laguna, Spain; [17]Hospital del Mar-Institut Municipal dInvestigaci Mdica (IMIM), Universitat Pompeu Fabra, Barcelona, Spain; [18]Vermont Cancer Registry, Burlington, VT; [19]Maine Cancer Registry, Augusta, ME; [20]Dartmouth Medical School, Hanover, NH; [21]Department of Urology, Washington University School of Medicine, St. Louis, MO; [22]National Institute for Health and Welfare, Helsinki, Finland; [23]Department of Epidemiology, Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, MA

A recent genome-wide association study (GWAS) of bladder cancer identified the UGT1A gene cluster on chromosome 2q37.1 as a novel susceptibility locus. The UGT1A cluster encodes a family of UDP-glucuronosyltransferases (UGTs), which facilitate cellular detoxification and removal of aromatic amines. Bioactivated forms of aromatic amines found in tobacco smoke and industrial chemicals are the main risk factors for bladder cancer. The association within the UGT1A locus was detected by a single nucleotide polymorphism (SNP) rs11892031. Now, we performed detailed resequencing, genotyping and imputation in this region. We clarified the original genetic association detected by rs11892031 and identified an uncommon SNP rs17863783 that explained and strengthened the association in this region (allele frequency 0.014 in 4,035 cases and 0.025 in 5,284 controls, OR=0.55, 95%CI=0.44-0.69, p=3.3x10-7). Rs17863783 is a synonymous coding variant Val209Val within the functional UGT1A6.1 splicing form, strongly expressed in the liver, kidney and bladder. We found the protective T allele of rs17863783 to be associated with increased mRNA expression of UGT1A6.1 in in-vitro exontrap assays and in human liver tissue samples. We suggest rs17863783 may protect from bladder cancer by increasing the removal of carcinogens from bladder epithelium by the UGT1A6.1 protein. Our study shows an example of genetic and functional role of an uncommon genetic variant in a complex human disease such as bladder cancer.

#32

Identification and characterization of novel microRNAs in the prostate cancer susceptibility locus 11q13

Hye Kyung Kim, Charles C. Chung, and Stephen Chanock
Laboratory of Translational Genetics (LTG), DCEG, NCI, NIH

Genome wide association studies (GWAS) have led to the identification of a series of common genetic variants associated with increased risk for cancers. One of such variant in chromosome 11 (rs10896449; Chr11q13:68994667 hg19) was shown to be significantly associated (P = 1.76 x 10-9) with increased susceptibility to prostate cancer. This marker resides ~67 kb downstream of MYEOV and ~135 kb upstream TPCN2 but did not show significant association with either of these genes or any other a high profile candidate gene. Consequently, the absence of recognizably functional transcripts in this intergenic region made it difficult to interpret how variants in this locus could be linked to increased cancer risk. Micro RNAs have recently emerged as important contributors to tumorigenesis. Also, many miRNA genes are found in genomic instable sites, which coincidentally also correspond to sites identified in GWAS for cancer susceptibility loci. Because 11q13 is a well documented fragile site, here we assessed the possibility of miRNA gene expression at 11q13. To this end, first, we analyzed the genomic DNA of Chr11: 68,868,292 ~69,067,087 for evolutionally conserved regions using the UCSC genome browser. Next, we tested these conserved regions for potential miRNA genes using a combination of the web-based miRNA detection programs, miRFinder and One-Class MiRNAfind, and excluded potential pseudo miRNA using the MiPred analysis program. As a result, we identified sixteen potential miRNAs. Among these, five miRNA were actually validated when tested in a panel of 20 different tissue samples using custom-made stem-loop RT-TaqMan miRNA asssays. Importantly,fine map analysis of the 11q13 region placed these novel miRNA genes into 11q13 GWAS signals, which suggest their potential association and contribution to increased cancer risk. Further characterization of these novel miRNA, their genetic variations, and expression profiles will be discussed.

#33

Towards Evaluating the Effect of Laser Dissection Approaches on Breast Cancer Proteome Profiles

Sumana M. Dey[1],Qiang Du[2], Adele Blacker[2], Hanson Jeffrey[2], Michael A Tangrea[2], Sanford P Markey[3], Michael R. Emmert-Buck[2], Donald J. Johann, Jr[1]
[1]Medical Oncology Branch, CCR, NCI; [2]Pathogenetic Unit, Laboratory of Pathology, CCR, NCI; [3]Neuroscience, NIH

The purpose of this study is to evaluate the effect of a variety of laser dissection approaches on the revealed proteome of human tumors. Our working hypothesis is that by using a laser dissection approach, the subsequent revealed proteins and pathways will show further enrichment for drug targeting. Two breast cancer tumors were used in this study. Both breast tumors were formalin fixed and paraffin embedded (FFPE), estrogen positive (ER+) invasive ductal carcinomas (IDC). Additionally, both tumors were a pathologic stage II and histologic grade II. All samples underwent a similar tissue proteomic prep and were further processed and analyzed using a solution-based liquid chromatography tandem mass spectrometry approach (LC-MS/MS). Thin tissue scrapes were compared to a variety of laser microdissected approaches. The various methods used for laser microdissection in this study involved: i) a manual LCM approach focused on the isolation of epithelial cells versus, ii) an automated laser dissection approach focused on epithelial cell collection versus, iii) an automated laser dissection approach that also utilized immuno staining of cytokeratin. In all laser dissection samples, approximately 60,000 cells were captured for subsequent analysis. All tissue specimens underwent an identical laser dissection prep utilizing a standard Hematoxylin and Eosin (H & E) approach. Expression Microdissection (xMD) using cytokeratin was used for the automated approaches both with and without immuno staining for cytokeratin. The results to date will be presented and contrasted.

#34

Uncharacterized protein C7orf43 binds to the TRAPPII binding domain of Rab8 GEF, a key protein in primary cilium assembly

Jeffery T. Gray, Vijay Walia, Amy Xu, and Christopher J. Westlake
Laboratory of Cell Development and Signaling, NCI-Frederick, NIH

The primary cilium is an organelle that plays a key role in sensory and signaling events. Defects in the formation and/or function of the primary cilium have been linked to many disorders including retinal degeneration, polydactyly, and various forms of cancer. Assembly of the primary cilium is mediated by a Rab cascade. Rab family proteins are small monomeric GTPases that regulate vesicle budding, motility, and fusion through the recruitment of various effector proteins. The downstream functions of Rab proteins depend on their transition to an active GTP-bound state via guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP for GTP. Our previous work has shown that Rab8 GEF (Rabin8) is transported to the centrosome via Rab11 vesicles and activates Rab8 to induce ciliogenesis. Using hTERT RPE cells, we have shown that primary cilium assembly is complete in approximately 1.5 hours following induction by serum withdrawal. As it has been shown that vesicular trafficking events are essential for ciliogenesis, we propose that Rabin8-interacting proteins may be crucial for these membrane transport processes. Our previous work identified Rabin8-associated proteins by localization and affinity purification (LAP)-tagging of Rabin8. LC-MS analysis of purified Rabin8 shows interaction with the transport protein particle (TRAPPII) complex, which aids in preciliary vesicle tethering and targeting. In addition, LC-MS data show interactions with three other proteins: UBR-5, FASN, and C7orf43. Of particular interest is C7orf43, a protein that encodes an uncharacterized protein. C7orf43 shares weak homology to Sec16A, a regulator of vesicle budding. Co-immunoprecipitation (IP) experiments and LC-MS analyses of Rabin8 fragments have shown that C7orf43 specifically binds to the first 140 amino acids of Rabin 8, the same domain previously shown to interact with the TRAPPII complex. We are further investigating the connection between C7orf43 and TRAPPII-mediated trafficking of Rabin8 during primary cilium membrane assembly.

Cancer Models, Metastasis, Microbiology, and Biophysics

Oral Presentation

Early Resistance to Apoptosis in the Lung Contributes to the Pulmonary Metastatic Phenotype

James J. Morrow[1,2], Sung-Hyeok Hong[2], Ling Ren[2], Arnulfo Mendoza[2], Ananth Eleswarapu[1,2], Chand Khanna[2]

[1]Howard Hughes Medical Institute-National Institutes of Health Research Scholars Program, Bethesda, MD; [2]Tumor Metastasis and Biology Section, Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Metastasis, the primary cause of death among cancer patients, consists of a complex sequence of events involving the tumor, the microenvironment, and the host. The process of metastasis is, however, inefficient, with only a small percentage of the cancer cells arriving at a given secondary organ persisting to form metastatic lesions. Pulmonary metastasis, specifically, has been shown to be highly inefficient by the previous work of Ruth Muschel and Ann Chambers. However, the timing of events determining the efficiency of metastasis to the lung remains poorly defined. We hypothesize that resistance to cell death during early vulnerable periods is a primary determinant of the highly metastatic phenotype in cancer cell populations with a proclivity for lung metastasis. To explore this hypothesis, we used a mouse tail vein injection pulmonary metastasis model to examine both sarcoma [K7M2, K7M2 AS ezrin 1.46 (AS1.46), and 1.52 (AS1.52)] and breast cancer (4T1 and 67NR) cell lines, two cancer types known to metastasize to the lung. Using ex vivo single cell video microscopy (SCVM) to track GFP-labeled cells, we were able to show that these cells undergo significant attrition upon their arrival to the lung. Surprisingly, we found that high and low metastatic cell lines can be distinguished by their metastatic efficiency as early as 6 hours after arrival at the lung; 15% of the highly metastatic K7M2 cells persisted in the lung at 6 hours compared to 4% of the less metastatic AS1.46 and AS1.52 cell lines. This indicates a relevant vulnerable time frame much earlier than that identified in previous studies. In addition, we showed using an ex vivo terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and an in vivo fluorescent marker of activated caspases (SR FLIVOTM) that low metastatic breast and sarcoma cell lines undergo apoptosis at more than twice the rate of their highly metastatic counterparts within 6 hours of arriving to the lung ($P<0.05$). Finally, we were able to reduce the rate of caspase activation by 44% in low metastatic cells 2 hours after their arrival to the lung using the broad-spectrum caspase inhibitor, Q-VD-OPh. Ongoing studies seek to dissect the relevant microenvironmental stresses and apoptotic pathways in these cancer cell populations that contribute to the phenomenon of metastatic inefficiency in the lung during this critical 6 hour window which we have defined.

Oral Presentation

Enforced Expression of Lin28b Results in Peripheral T Cell Lymphoma In Vivo

Sarah H. Beachy[1], Masahiro Onozawa[1], Yang Jo Chung[1], Deborah Silverman[1], Chris Slape[2], Sven Bilke[1], Princy Francis[1], Marbin Pineda[1], Robert L. Walker[1], Paul Meltzer[1], and Peter D. Aplan[1]

[1]Genetics Branch, CCR, NCI, NIH, Bethesda, MD; [2]Central Clinical School, Monash University, Melbourne, Australia

Lin28a (previously Lin28) and Lin28b are mammalian homologues of lin-28, a heterochronic gene in C. elegans initially expressed during early larval stages and expressed at decreased levels in later developmental stages. Work from other groups demonstrated that induced expression of LIN28A, in collaboration with NANOG, OCT4 and SOX2 can reprogram human fibroblasts to acquire pluripotency, providing additional evidence for a positive correlation between LIN28A expression and a more immature stem cell-like state. Additionally, LIN28A and LIN28B have been noted to be overexpressed in several tumor types; overexpression of LIN28A and LIN28B promotes the degradation of the let-7 family of microRNAs (miRs), which are known to target oncogenes such as Myc and Ras. In order to explore the oncogenic properties of Lin28b in vivo, we generated a Lin28b transgenic mouse that targeted expression of the transgene to hematopoietic tissues under the control of Vav regulatory elements. We found that clinically healthy Lin28b mice exhibited aberrant thymic architecture and retention of thymocytes that was correlated with peripheral blood lymphopenia (a 2.6-fold decrease in circulating lymphocytes) due to decreased numbers of CD4+ and CD8+ cells. Despite the decrease in total CD4+ and CD8+ T cells, we found more than a 3-fold increase in peripheral CD4+ and CD8+ effector memory T cells (CD44hiCD62Llo) in the Lin28b transgenic mice. Deep sequencing of miRs revealed a 2 to 5-fold downregulation of let-7 family members, including let-7d, g, f, i and miR-98 in the thymus of clinically healthy Lin28b transgenic mice. At approximately 14 months of age, Lin28b mice began to develop an aggressive, lethal, peripheral T cell lymphoma (PTCL), characterized by widespread infiltration of parenchymal organs with a mixed infiltrate of inflammatory cells and malignant CD4+ T cells. Clonal Tcrb gene rearrangements were detected in the lymphomas, and transplanted malignant cells formed tumors in immunodeficient Scid mice. Evidence of clonal T cell expansion, documented by clonal or oligoclonal Tcrb gene rearrangements, was also detected in some mice prior to the onset of clinically observable PTCL. The transgenic mice with PTCL had clinical signs consistent with a chronic inflammatory condition, such as eosinophilia, anemia, pleural effusions and ascites. A previously reported inflammatory pathway linked to malignant transformation and involving Lin28b was also evaluated. The Lin28b lymphomas overexpressed genes in that pathway, including Il6, Myc, and activated Nfκb. Analysis of a publically available dataset indicated that Lin28b was overexpressed by 8-fold in a set of PTCL patient samples compared with activated CD4+ cells, demonstrating that overexpression of Lin28b can be linked to human as well as murine PTCL. These findings demonstrate in vivo evidence for an oncogenic function of Lin28b and provide a model for further study of PTCL biology as well as a preclinical platform useful for the evaluation of therapeutic approaches for PTCL.

Oral Presentation

Biochemical and functional interaction of two metastasis suppressors, Nm23-H1 and Gelsolin, in breast carcinoma cells.

Natascia Marino[1], Jean-Claude A. Marshall[1], Joshua Collins[1], Ming Zhou[2],Timothy Veenstra[2], Patricia S. Steeg[1]

[1]Womens Cancers Section, Laboratory of Molecular Pharmacology, CCR, NCI, NIH, Bethesda, MD; [2]NCI, NIH, Frederick, MD

Metastasis suppressors are a relatively new class of proteins that regulate and inhibit the metastasis formation without modifying or blocking the primary tumor growth. Nm23 was the first metastasis suppressor gene to be identified, although the biochemical basis for Nm23s anti-metastatic properties remains to be fully elucidated. Therefore we undertook a mechanistic study of Nm23 binding partners hypothesizing that Nm23s interaction with other proteins may play a role in regulating metastasis. Co-immunoprecipitation assays and mass spectrometry analysis identifyed a new interaction with the metastasis suppressor Gelsolin. Gelsolin is an actin binding protein involved in actin-cytoskeleton remodeling through its actin-severing, -capping and newly discovered -nucleating activities. Two-way co-immunoprecipitations were performed in 4T1 cells and several human breast carcinoma cell lines (MCF7, MDA-MB-231, MDA-MB-435) to confirm the association. The potential interaction of Nm23-H1 and Gelsolin was investigated by overexpressing each protein, or the combination, in both murine (4T1) and human (MDA-MB-231) breast carcinoma cell lines. Nm23-H1 overexpression, as well as Gelsolin overexpression, reduced motility in Boyden chambers by 63.1% and 29,6% respectively as compared to vector clones. The co-overexpression of both genes enhanced the reduction in motility by 82.3% ($p<0.001$). Invasion assays showed similar trends The data demonstrate an additive interaction of two metastasis suppressors in motility and invasion. Since Gelsolin is involved in the actin-cytoskeleton organization, regulating actin polymerization and depolimerization, we investigated the role of Nm23 in these processes. In both MDA-MB-231 and 4T1 cell lines, Nm23-H1 overexpression did not modify the actin-polymerization as compared the vector clones, however an increase in F-actin depolimerization was observed. Studies analyzing the in vivo effect of the co-overexpression of the two proteins are ongoing. The data provide an insight into the interaction of metastasis suppressors and may represent a step for the understanding of their biological mechanisms.

Oral Presentation

IKKα links inflammation and tumorigenesis in lung squamous cell carcinoma

Qun Jiang[1], Zouxiang Xiao[2], Jami Willette-Brown[2], Timothy Back[1], Feng Zhu[2], Mahesh Datla[2], Robert H. Wiltrout[1], Yinling Hu[2]

[1]Experimental Therapeutics Section; [2]Inflammation and Tumorigenesis Section, Laboratory of Experimental Immunology, Cancer and Inflammation Program, CCR, NCI, NCI-Frederick, Frederick, MD

Lung cancer is the leading cause of cancer deaths worldwide. Although various genetically engineered and chemical induced lung adenocarcinoma mouse models have been established, spontaneous lung squamous cell carcinomas (SCC) mouse models are very rare. IkB Kinase subunit α (IKKα) mutations have been reported in many types of human cancer. Our recent study established a mouse model of lung squamous cell carcinoma (SCC) by inactivating IKKα in vivo, and found EGFR/Ras/p38 MAPK pathways were activated and played important roles in lung SCC tumorigenesis. However, IKKαs attributes relating to linkage between inflammation and lung SCC have not yet been well-defined. We here show that inactivation of IKKα in mice causes systemic whole-life chronic inflammation, which consequently promotes lung SCC initiation and progression. In mice with mutated IKKα, CD4 T cells, dendritic cells, and macrophages are all significantly increased in the spleen and lung. Proinflammatory cytokines and chemokines are also remarkably elevated in the lungs of these mice. An M2 macrophage phenotype is observed only in the lungs of old IKKα mutant mice, not in younger mice, suggesting the M1/M2 polarization may be driven by the proinflammatory microenvironment in these mice. Production of Th2 cytokines and IL-10 are elevated in CD4 T cells from the lungs of mice with mutated IKKα, and may contribute to M2 polarization during the chronic inflammation. In addition, depletion of macrophages in these mice can significantly reduce Th2 cytokine production, oxidative DNA damage, Ras/p38 MAPK pathway activation and completely shut down SCC development in the lungs, suggesting their important roles in inflammation-associated SCC tumorigenesis. Taken together, our study generates, for the first time, a spontaneous mouse model of inflammation-associated lung SCC, and suggests that IKKα plays important roles in linking inflammation and carcinogenesis of lung squamous cell carcinoma.

Oral Presentation

E3 ubiquitin ligase activity of human BRCA1 is necessary for its tumor suppression function.

Suhwan Chang, Betty K Martin, Stacey Stauffer and Shyam K Sharan

Mouse Cancer Genetics Program, CCR, NCI-Frederick, Frederick, MD

E3 ligase function of BRCA1 has been shown to play an important role in DNA damage repair as well as in genomic integrity. Despite its important biochemical function, the E3 ligase activity of BRCA1 has been reported to be dispensable for tumor suppression in mice. Here we describe that the E3 ligase activity of human BRCA1 is essential to support survival of mouse embryonic stem cells as well as to exert its tumor suppressor function in vivo. We examined the functional consequences of two missense mutations in BRCA1, H41R and I26A, which are predicted to disrupt its E2 interaction while maintaining the structural integrity of RING domain. Using an embryonic stem cell based assay we demonstrated that these mutants fail to rescue the lethality of Brca1 null ES cells. Moreover, transgenic expression of human I26A mutant BRCA1 in Brca1+/-;p53+/- and Brca1cko/cko;p53cko/cko;K14-Cre mouse mammary tumor model showed similar tumor incidence or tumor free survival compared to non-transgenic control mice. Biochemical analysis in tumors and primary tumor cell lines showed that the human I26A BRCA1 expression failed to restore the decreased H2A ubiquitination or up-regulation of microsatellite expression compared to the expression of another BRCA1 variant, R1699Q, which retains E3 ligase function. Finally, the co-segregation analysis of H41R mutation in a family with a history of breast cancer strongly suggests that this mutation is associated with increased breast cancer risk. Taken together, our study demonstrates the essential role of E3 ligase activity of human BRCA1 for its tumor suppression function.

Oral Presentation

Functional Characterization of Rab31 Gene in Pancreatic Cancer Cell Tumorigenesis

Akiko Chiba, Vijay Walia, Laufey Amunadottir, and Chris Westlake

Laboratory of Cell and Developmental Signaling, NCI-Frederick

Small GTPase proteins of the Rab family play an important role in vesicular trafficking. Differential regulation of Rabs has been linked to human genetic and acquired diseases including neurodegenerative disorders, diabetes, and cancer. Examination of ~650 membrane trafficking regulator (Membrome) expression via microarray and RNA seq demonstrated that Rab31 is expressed at higher levels in pancreatic tumors and cell lines. Rab31 functions in the biosynthetic pathway at the trans-Golgi. Recently, Rab31 was reported to be a poor prognostic factor in breast cancer patients, however, how Rab31 functions in tumorigenesis is not known. We performed siRNA ablation experiments and found that Rab31 functions in Panc1 cell migration and invasion, but was dispensable for cell proliferation and apoptosis. To further study Rab31 roles in pancreatic cancer cell function, a Tet-inducible shRNA expressing Panc1 cell line was generated using lentiviral vectors for *in vitro* (cell culture) and *in vivo* (xenograft) experiments. Finally using immunoprecipitation and mass spectrometry, we have determined that Rab31 binds to Rac1 and Cdc42, regulators of cell migration. Together our results suggest that elevated Rab31 levels may enhance pancreatic cancer tumorigenesis via association with key regulators of cell migration and invasion.

#35

Increased Mutation Frequency induced by Oxidative Stress in the NUP98-HOXD13 MDS mouse model

Yang Jo Chung[1], Carine Robert[2], Sheryl M. Gough[1], Feyruz V. Rassool[2] and Peter D. Aplan[1]

[1]Genetics Branch, Center for Cancer Research, NCI, NIH; [2]Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD

The myelodysplastic syndromes (MDS) comprise a group of hematologic disorders characterized by ineffective hematopoiesis, dysplasia, and transformation to acute myeloid leukemia (AML) in a subset of cases. The causative agents for de novo or therapy-related MDS (t-MDS) include exposure to known genotoxins, such as anti-cancer chemotherapy, or ionizing radiation. It is known that MDS is associated with chromosomal aberrations including deletions, amplifications, inversions, and translocations. However, the molecular mechanisms that lead to progression of MDS to AML have not been completely elucidated. Recently, several studies have reported that oxidative stress contributes to disease progression of MDS. Reactive oxygen species (ROS) play a role in regulating several biologic phenomena including activation of signaling pathways in response to cytokines, and the gene expression pattern induced by this signaling. ROS is also known to induce oxidative DNA damage, which can lead to activation of proto-oncogenes or inactivation of tumor suppressor genes. A mouse model for MDS, generated by expression of a NUP98-HOXD13 (NHD13) fusion gene, was exploited to investigate ROS levels. Lineage negative (Linneg) bone marrow mononuclear cell (BMNC) from NHD13 mice with MDS had a 6.0 fold increased level of ROS compared with wild-type (WT) Linneg BMNC. In order to address potential mechanism(s) leading to this increased ROS, a Real-Time Quantitative PCR array, which profiles the expression of 84 genes related to oxidative stress, was utilized and the data demonstrated down regulation of Myeloperoxidase (Mpo; -9.9 fold), Eosinophil peroxidase (Epx; -8.8 fold) and Lactoperoxidase (Lpo; -5.2 fold) in the Linneg BMNC from NHD13 mice. The increased ROS level in NHD13 Linneg BMNC was associated with a 2 fold increase in G2/M phase cells along with increased expression of p16INK4A (43 fold) and p21 (10 fold), possibly to allow for DNA repair at cell cycle checkpoints. In order to investigate genomic DNA damage by ROS, immunocytochemistry for γH2AX foci was performed and demonstrated a 2 fold increase in γH2AX foci in the NHD13 BMNC compared to WT BMNC. To determine if mis-repair of the ROS-induced DNA damage led to mutations of genomic DNA in vivo, mutation frequencies (MF) were determined using the Big Blue cII mutation detection assay. Big Blue mice are transgenic mice that have a λLIZ shuttle vector, which is derived from the coliphage lambda. Selection of mutation is based on the ability of the λ phage to multiply through either the lytic or lysogenic cycle in E. coli host cells. Big Blue mice were crossed to NHD13 mice, and NHD13/Big Blue mice were compared with littermate controls that were positive for the BigBlue transgene but negative for the NHD13 transgene. BMNC from the NHD13/BigBlue mice showed a 1.8-fold increased MF compared to control BMNC (p value = 0.0371). Mutations were comfirmed by sequencing the cII target gene, and revealed that the NHD13/BigBlue mice had a 3-fold higher frequency of frameshift mutations compared to the control animals. These results suggest that the oxidative stress induced by the NHD13 fusion may contribute to disease progression of MDS to AML through DNA damage and mutation.

#36

Transplant-associated alloreactivity provides the "first hit" in a negative regulatory sequence that results in graft-versus-tumor resistance

Jessica Shand[1], Haiying Qin[2], Christian Capitini[2] and Terry Fry[2]

[1]Johns Hopkins-National Cancer Institute Combined Fellowship, Baltimore and Bethesda, MD; [2]Pediatric Oncology Branch, NCI, CCR, NCI

Relapse following allogeneic transplant is the leading cause of treatment failure in acute lymphoblastic leukemia (ALL). Preclinical and clinical data suggest that ALL is particularly resistant to the graft-versus-leukemia (GVL) effect. We hypothesize that the alloimmune environment initiates a sequence of negative regulation that ultimately renders alloreactive CD8 susceptible to tumor immune evasion. A preclinical murine model of allogeneic transplant was developed in which donors and recipients are mismatched to control tissue expression of the male minor histocompatibility antigen HY. Following lethal irradiation and T-cell depleted bone marrow transplant, recipients received HY-specific CD8. As expected, broad expression of HY (female $\rightarrow$ male) produces clinical GVHD in response to HY-specific DLI. Unexpectedly, HY-directed alloreactivity provides no protection when male recipients are challenged with an HY-expressing tumor (MB49). Prior to tumor challenge, nearly 100% of HY-specific CD8 recovered from secondary lymphoid organs express high levels of the negative regulator PD-1, have persistently elevated proportions of effector memory cells and do not proliferate upon subsequent HY antigen stimulation, consistent with T-cell dysfunction. Polyclonal donor CD8 reconstituted independently of the DLI do not express PD-1, suggesting that the phenomenon is antigen-specific. Interestingly, tumor protection is partly restored by hematopoietic restriction of HY, despite HY-specific CD8 also expressing high levels of PD-1. However, significantly fewer of these CD8 coexpress Tim-3, a second immune checkpoint regulator. We therefore propose that alloreactivity-induced PD-1 expression represents the first step in sequence of cooperative negative regulatory events that require input from the inflammatory marrow niche and tumors that express inhibitory ligands. Studies are ongoing to address functional and genomic differences in T-cells recovered from these settings, while incorporating an HY-expressing murine pre-B cell leukemia (E2A-PBX) that uniformly expresses the PD-1 ligand in an attempt to identify the optimal timing and target(s) of inhibitory blockade in the alloreactivity-induced dysfunction sequence.

#37

The role of nitric oxide in the metastatic colonization of the lung in osteosarcoma

Michael Lizardo, Ling Ren, and Chand Khanna

Pediatric Oncology Branch, CCR, NCI

The presence of pulmonary metastases in pediatric osteosaroma patients is associated with poor outcome. This clinical problem underscores the need to develop a better understanding of metastasis progression, and to develop novel anti-metastatic therapeutics. Our research efforts have focused on the stresses that metastatic tumour cells must overcome to survive and grow in the lung. In particular, endothelial cell-derived nitric oxide (NO) has been shown to induce apoptosis in metastatic cells. To understand how highly aggressive metastatic cells adapt to NO stress verses poorly metastatic cells, our research utilizes a pair of related murine osteosarcoma cells with contrasting metastatic phenotypes: highly metastatic K7M2 cells and low metastatic K12 cells. To assess how these cell lines respond to NO in vitro, we have performed cytotoxicity and metabolic activity assays in the presence of the NO donor compound, diethylenetriamine NONOate (DETA/NO). In our cytotoxicity assays, we find that K12 cells are ~2.3 fold more sensitive to DETA/NO compared to K7M2 cells. Furthermore, the metabolic activity of K12 cells in the presence of DETA/NO was significantly decreased compared to K7M2 cells (unpaired t-test, $p < 0.001$). To ascertain whether NO in the lung microenvironment is a factor that contributes to the metastatic inefficiency of K12 cells, we assessed the ability of K12 cells to grow in the pulmonary metastasis assay (PuMA) in the presence or absence of L-NG-Nitroarginine methyl ester (L-NAME), a competitive inhibitor of endothelial nitric oxide synthase. We find that in the presence of L-NAME, K12 cells grew larger lesions in lung tissues compared to vehicle treated group. Taken together, these results suggest that the ability to adapt to NO stress is what distinguishes high and low metastatic murine osteosarcoma cells. Investigations are ongoing to elucidate the cellular mechanisms by which highly metastatic cells adapt to NO stress.

#38

Identification of N-end rule substrates in Escherichia coli

Matthew A. Humbard, Serhiy Surkov, and Michael R. Maurizi

Laboratory of Cell Biology, CCR, NCI

The N-end rule is a universally conserved mechanism for the targeting and degradation of proteins by energy-dependent proteases. Destabilizing amino acids at the N-terminus (N-degrons) stimulate degradation of substrates. In Escherichia coli, ClpS is a small adapter protein that interacts with the energy dependent protease complex ClpAP and is responsible for the delivery of N-end rule substrates for degradation. ClpS interacts with the N-terminus of a protein when the terminal amino acid is a non-canonical hydrophobic residue (Phe, Trp, Try, or Leu). To become a substrate of the N-end rule pathway, proteins must be modified at the N-terminus to convert their canonical N-termini to one recognized by ClpS. This conversion can occur through a cleavage by a peptidase and/or modification by the enzyme Aat, which adds a leucine or phenylalanine to the free α-amine on the N-terminus of the protein when the N-terminal residue is positively charged (Lys or Arg). While much is known about the biochemistry of the protease and the presentation of substrates by the adapter protein, how substrates are generated and the identities of those substrates have largely remained a mystery. By isolating proteins that interact with ClpS, we identified several dozen novel substrates of the N-end rule. Our findings reveal roles for the N-end rule in translation, DNA replication, transcription, membrane permeability, and motility. Additionally, N-terminal sequencing of substrates reveals that most were processed by peptidases and some were further modified by Aat. Aat or ClpS substrates are generated by multiple peptidases. YfbL, a peptidase of previous unknown function, appears to be responsible for the creation of several Aat-independent substrates of ClpS including Dps, an abundant substrate of this system. Identification and characterization of these peptidases will further our understanding of the role the N-end rule plays in bacterial physiology.

#39

A GSA-NMP1 conjugate: An NIR-activatable fluorescence imaging agent to detect peritoneal ovarian cancer metastases

Vinita M. Alexander[1], Kohei Sano[1], Zhanqian Yu[1], Takahito Nakajima[1], Peter L. Choyke[1], Marcin Ptaszek[2], and Hisataka Kobayashi[1]

[1]Molecular Imaging Program, CCR, NCI, Bethesda, MD; [2]University of Maryland Baltimore County, Baltimore, MD

PURPOSE: There is a need to improve detection of peritoneal ovarian cancer metastases during surgery. Previous designs of activatable GSA-fluorophore probes have used only visible range dyes conjugated to GSA. However, activatable agents emitting fluorescence in the NIR range are advantageous because NIR photons have deeper in vivo tissue penetration and lower background autofluorescence than those emitting in the visible range. An NIR-activatable GSA fluorophore pair can be synthesized using the dye NMP1. This NMP1 dye has two unique absorption peaks (in green and NIR wavelength ranges) with a single NIR emission peak of 780nm. NMP1 thus has two different Stokes shifts that have the potential to allow separate imaging of tumor nodules both at the surface and at depths below the surface. DESIGN: GSA was conjugated with 2 NMP1 molecules to create a self-quenching complex (GSA-NMP1). The activation magnitude of GSA-NMP1 was validated by adding 10% SDS. Flow cytometry, fluorescence microscopy, and in vivo spectral fluorescence imaging were carried out to compare GSA-NMP1 with GSA-IR800 (an always on control agent with similar emission to NMP1) in terms of intracellular activation and abilities to allow detection of small ovarian cancer implants in mice models. Sensitivity and specificity of GSA-NMP1 for implant detection were determined by co-localizing NMP1 emission spectra with red fluorescent protein expressed constitutively in SHIN3 tumor implants. Image processing in Java was used to subtract spectral images, with resulting images theorized to show tumor nodules at a depth below the surface. RESULTS: The fluorescence signal of GSA-NMP1 can be activated ~100-fold and was specifically yielded after internalization into SHIN3 cells 3h after incubation. Submillimeter ovarian cancer implants in the peritoneal cavity were clearly detectable in vivo with spectral fluorescence imaging. Among 555 peritoneal lesions, the sensitivity and specificity for GSA-NMP using NIR and green excitation light, were 75%/92% and 91%/92% for lesions ~0.8 mm or greater in diameter, respectively. Image processing suggests that nodules at a depth below the small bowel are imaged only with GSA-NMP1 by subtraction of the two excitation images. CONCLUSIONS: GSA-NMP1 is useful in imaging peritoneal ovarian cancer metastases, located both superficially and deep in the abdominal cavity.

#40

Analysis of collective forces involved in epithelial sheet migration

Rachel Lee[1], Michael Weiger[2], Christina Stuelton[2], Carole Parent[2], and Wolfgang Losert[1]

[1]Department of Physics, University of Maryland, College Park; [2]Laboratory of Cellular and Molecular Biology, CCR, NCI

Epithelial cells typically form sheets of tightly associated cells that facilitate collective cell migration in contrast to single cell migration. However, during progression of epithelial tumors, cells acquire the ability to leave the epithelial sheet, invade surrounding tissue and metastasize to distant sites. Despite the dire prognosis of metastatic disease and the accepted notion that changes in the migratory capacity of tumor cells contribute to metastatic disease, it is not yet well understood how the migratory properties of metastatic tumor cells differ from those of non-metastatic tumor and epithelial cells. Specifically, no quantitative parameters are available to analyze the transition of collective sheet migration to single cell migration and to predict metastatic behavior. Our interest is to develop a mechanics framework for collective migration based on the balance of forces the cells experience during migration. Current investigations will infer the balance of cell-cell adhesion, cell-substrate adhesion, contractile forces, and protrusive forces from the dynamics of cell shapes (individual and collective). As an in vitro model system for tumor progression we chose four cell lines of different in vivo malignancy (epithelial, pre-malignant, tumorigenic, and metastatic) and recorded cell sheets during an unconstrained migration assay using live cell imaging. Initial analysis focuses on the movement of the leading edge. We found that the four cell lines show noticeably different behavior in their edge dynamics. For example, the epithelial and pre-malignant cells move in a collective, directed manner while the tumorigenic and metastatic cells are more individualistic. We now continue to analyze migratory patterns and shape by exposing the cells to inhibitors selected to affect cell adhesion and cytoskeletal integrity. Determining the balance of forces between cell lines and how inhibitors affect the balance will allow for the development of more effective diagnosis techniques and therapies for metastatic cancers.

Immunology II

Oral Presentation

IL-10 from Tumor T Regulatory Cells is Induced by Type I Interferon and Limits the Local Th-17 Response

C. Andrew Stewart[1], Hannah Metheny[1], Amiran Dzutsev[1], Miranda Hanson[1], Xin Chen[1], Werner Muller[3], Axel Roers[4], Christopher Karp[2], Giorgio Trinchieri[1]

[1]Cancer Inflammation Program, CCR, NCI-Frederick, Frederick MD; [2]Cincinnati Children's Hospital Research Foundation and the University of Cincinnati College of Medicine, Cincinnati, OH; [3]University of Manchester, Manchester, UK; [4]Technische Universitat Dresden, Dresden, Germany

The tumor microenvironment is characterized by inflammation that contributes to tumor growth and limits immunotherapy through immunosuppression such as that mediated by interleukin-10. To understand the role of IL-10 in the tumor microenvironment, we examined the cellular sources of IL-10, the signals required for IL-10 production, and how the absence of IL-10 affects immune regulation within the tumor. Using IL-10GFP (VERT-X) reporter mice and T cell conditional IL-10 knockout mice we show that T regulatory cells (Treg) are the predominant source of IL-10 in the tumor microenvironment. Approximately half of tumor Treg from a variety of tumor models expressed IL-10, contrasting with low frequencies of IL-10 positive Treg in normal tissues. Microarray analysis and screening of knockout mice was performed to identify regulatory pathways involved in expression of IL-10 by tumor Treg. IL-10 expression by these cells was associated with expression of activation-associated genes including the transcription factor Maf. MyD88-dependent and IL-12 family-dependent pathways were not required for Treg IL-10, but STAT1 and IFNAR1 were critical inflammatory mediators for Treg accumulation, activation and IL-10 production. To assess the role of Treg IL-10, we analysed T cell-conditional IL-10 knockout mice. In the absence of T cell IL-10, higher frequencies of CD4+ T cells, and specifically pro-angiogenic Th17 cells were observed in the tumor microenvironment. Our data indicate that type I interferons, through IFNAR1, are required for activation of tumor Treg and their expression of IL-10. This IL-10 plays a specific role in balancing inflammation in the tumor by limiting numbers of pro-angiogenic Th17 cells. As blockade of IL-10 signaling is an important constituent of cancer immunotherapy protocols that are currently under development, controlled intervention with agents that selectively inhibit IL-10 signaling during the short window of immunotherapy will be required to maintain IL-10s anti-tumor effect before and after treatment.

Oral Presentation

Tumor-Induced CD79 Expressing Myeloid Cells Are Associated With The Metastatic Process In Mouse Models Of Breast Cancer

Dror Luger[1], Yu-an Yang[1], Asaf Raviv[1], Ellen S. Gamble[1], Doug Weinberg[1], Min-Jung Lee[2], Jane Trepel[2] and Lalage M. Wakefield[1]

[1]Cancer Biology of TGF-b Section, Laboratory of Cancer Biology and Genetics, NCI; [2]Medical Oncology Branch, NCI

Tumor-induced expansion of myeloid derived suppressor cells (MDSCs) is already well known. The current study was originally designed to elucidate the role that B cells may play in metastasis formation. For this purpose we used two well-established cell line models that were generated from the same tumor, the metastatic 4T1 and the non metastatic 67NR. Different B cell markers were examined in various organs at 28 days post inoculation of tumor cells to characterize the potential involvement of B cells. While the 67NR mice showed almost normal amount of mature B cells from the spleen and lung, that were positive for CD19, IgM and IgD, the metastatic 4T1 mice had significantly lower ratio of mature B cells. Surprisingly, we found an increased expression of the B cell marker CD79 on non-B cells in the spleens and lungs of mice with 4T1 tumors. Further analysis revealed that the tumor induce expansion of myeloid cells that express CD79b but no other markers of mature B cells. By FACS analysis we found that the myeloid CD79b can be recognized by the Ab clone HM79-11 but not by the conventional clone HM79-12 that recognize B cells only, suggesting that the CD79b may form several cell surface complexes that are restricted on the myeloid sub-population. This population can vary between 20 to 60% in the spleen and 50 to 90% in the BM of tumor bearing mice. Similar results were obtained in B- and T-cell-deficient SCID mice suggesting that the CD79+ myeloid cells did not derive from cells in the B-cell lineage. We have observed similar CD79+ myeloid cells in additional metastatic tumor models. In vivo treatment with Abs of both HM79-11 and HM79-12 resulted in significant depletion of myeloid cells by using the CD79-11 and not with the CD79-12 clone that showed more efficiency in depleting B cells. In vitro culture of splenocytes from tumor bearing mice with conditioned medium from the two cancer cell lines showed increased expression of CD79 on myeloid cells cultured with conditioned medium from the 4T1 cell line. Experiments to identify the secreted factor from 4T1 cells that upregulates CD79 expression are ongoing. Immunofluorescense analysis showed enhanced infiltration of CD79+ myeloid cells into the lungs of 4T1 but not 67NR tumor bearing mice. We further showed that myeloid cells that are positive for CD79 can be found in human breast cancer sections, which suggests this sub-population may be relevant to regulation of tumor progression in humans. We are currently testing the functional significance of this novel subpopulation of myeloid cells in the metastatic process.

Oral Presentation

Transgenic interleukin-6 enhances peripheral T cell survival and homeostasis

Anna S. Nam[1,2,3], Hilary Keller[1], Brett A. Linowes[1], Changwan Hong[1], Megan Luckey[1], and Jung-Hyun Park[1]

[1]Experimental Immunology Branch, NCI, NIH, Bethesda, MD; [2]University of Missouri School of Medicine, Columbia, MO; 3Howard Hughes Medical Institute, Bethesda, MD

Peripheral T cell homeostasis is dependent on interleukin-7 (IL-7), a common γ chain (γc) cytokine. However, IL-7 may not be unique in its ability to maintain peripheral T cells. IL-6which is not a γc cytokine but of the gp130 cytokine receptor familyhas been previously proposed to support peripheral T cell homeostasis. Specifically, in vitro studies have highlighted the role of IL-6 as a pro-survival cytokine. However, in vivo studies have been conflicting. Along this line, transgenic models have been limited by the development of polyclonal plasmacytosis in the thymus, lymph node and spleen of IL-6 transgenic (IL-6Tg) mice. To circumvent this problem, we generated IL-6Tg mice on an immunoglobulin heavy chain (IgH) deficient (IgH-KO) background, which rendered them B-cell deficient. These mice were tumor-free even after aging for more than 12 months. Importantly, we did not observe any defects in T cell development in the presence of transgenic IL-6. In fact, in young IgH-KO IL-6Tg mice (8-9 weeks old), thymocyte counts were significantly greater than that of the IgH-KO mice, suggesting that the overexpression of IL-6 promotes T cell development. In order to exclude the effect of thymic output, we examined aged mice (37-52 weeks old) whose thymic output was negligible. Indeed, total thymocyte numbers were comparable between aged IgH-KO and IgH-KO IL-6Tg mice. Thus, any changes in peripheral T cell function and survival in IL-6 transgenic mice are the direct effects of IL-6 on mature T cells. Here, we observed that IL-6 overexpression contributed to T cell survival as shown by significantly increased lymph node T cell numbers in lymph nodes. Interestingly, IL-6 did not show any selective effects on naive or activated/memory T cell populations. Moreover, T cell receptor excision circle (TREC) numbers in peripheral T cells were found to be decreased in IgH-KO IL-6Tg mice. To determine the mechanism underlying such pro-survival effects, we examined pro-survival molecule expression in IL-6 signaled cells and found that IL-6 failed to induce Bcl-2 and Bcl-xL but upregulated Pim-1 and Pim-2, which are potent inhibitors of the pro-apoptotic molecule Bad. In summary, we show that IL-6 can enhance peripheral T cell survival and maintenance in an in vivo system and that upregulation of Pim-1 and Pim-2 by IL-6 may be one of the mechanisms by which IL-6 contributes to T cell homeostasis.

Oral Presentation

A Pilot Trial of Wilms tumor 1 (WT1) Peptide-Loaded Allogeneic Dendritic Cell (DC) Vaccination and Donor Lymphocyte Infusions (DLI) for WT1-Expressing Hematologic Malignancies Relapsing after Allogeneic Hematopoietic Stem Cell Transplantation (alloHSCT)

Nirali N. Shah, MD,[1] David Loeb, MD, PhD,[2] Hahn Khuu, MD,[3] David Stroncek, MD,[3] Mark Raffeld, MD,[4] Cindy Delbrook, RN,[1] Kelly Richards, RN,[1] Kristin Baird, MD,[1] Jason Levine, MD,[1] Susan Leitman, MD,[3] Crystal L. Mackall, MD,[1] Terry J. Fry, MD,[1] and Alan S. Wayne, MD[1]

[1]Pediatric Oncology Branch, CCR, NCI, NIH; [2]Pediatric Oncology, Johns Hopkins University; [3]Cell Processing Section, Department of Transfusion Medicine, Clinical Center, NIH; [4]Laboratory of Pathology, CCR, NCI, NIH

Background: Treatment of relapse after allogeneic hematopoietic stem cell transplantation (AlloHSCT) remains challenging. The Wilms tumor 1 (WT1) gene product is a tumor-associated antigen expressed in many hematologic malignancies. This trial incorporates antigen-specific immunotherapy and allogeneic adoptive cell transfer for pediatric and adult patients with relapsed hematologic malignancies after AlloHSCT. The primary aims are to assess safety and feasibility of a novel vaccine designed to enhance the graft-vs-leukemia effect. Design: HLA-A2+ patients with WT1-expressing hematologic malignancies that have relapsed after AlloHSCT are eligible. Donor-derived DC vaccines are given every 2 weeks for 6 doses and DLI every 4 weeks for 3 doses. Peripheral blood monocyte-derived DCs are loaded with a combination of three HLA-A2 binding WT1 peptides. In attempt to enhance antigen presentation, peptides include an 11-mer TAT protein transduction domain. Study endpoints include toxicity, feasibility, antigen-specific immune response, and clinical response. Results: 4 patients, aged 9-19 years were treated, 3 with acute lymphoblastic leukemia (ALL) and one with Hodgkin lymphoma. Vaccines were successfully produced. All patients tolerated vaccine and DLI administration well. The most common adverse events were mild, reversible pain and pruritis at vaccine sites and delayed type hypersensitivity (DTH) skin test sites. One patient developed Grade I skin GVHD not requiring systemic therapy. All 3 patients with ALL demonstrated positive ELISPOT responses to WT1-peptides and positive DTH responses to the KLH control. 2/3 ALL patients showed DTH responses to the WT1-peptides. No immune response was observed in the patient with Hodgkin lymphoma. 1 patient with ALL remains in remission 10 months after initiation of therapy and 3 have died of disease. Median overall survival was 12 months. Conclusions: This novel allogeneic immunotherapy regimen is feasible, well tolerated and can induce tumor directed immune responses in patients with relapsed ALL following allogeneic stem cell transplantation. Accrual is ongoing.

Oral Presentation

Immunosuppression of the Host Immune System in Acute Lymphoblastic Leukemia: an insight into the role of PD-1

Paul P. Su[1,2], Chad Burk[1,2], William Fix[1], Hai-Ying Qin[1], Terry J. Fry[1]

[1]Pediatric Oncology Branch, CCR, NCI; [2]HHMI-NIH Research Scholars Program, HHMI, Bethesda MD

Acute lymphoblastic leukemia (ALL) is the most common cause of cancer related mortality in children. Immune-based therapies have been ushered to the forefront of investigation by promising effects of adoptively transferred ALL-specific T cells; but further improvements in therapy depend on a better understanding of the interactions between ALL and the host immune system. Using a newly developed murine model of precursor B-cell ALL, we showed that host CD4+ and CD8+ T cells exhibit a statistically significant increase in PD-1 expression in an ALL-progression dependent manner. This PD-1 expression pattern is seen even at the earliest infiltration of ALL and pervade as much as 80% of T cells with advanced disease. PD-1 represents an inhibitory axis of T cell regulation that has been implicated in T cell exhaustion and dysfunction in other tumor and chronic infection models. Further characterization of PD-1 expressing CD8+ cells by flow cytometry revealed a shift in T cell differentiation from nave (CD62L+ CD44-) to a differentiated effector memory phenotype (CD62L-CD44+). Finally, when we assessed the ability of T cells from leukemia bearing mice to degranulate, we showed impairments limited to PD-1+ expressing CD8+ T cells. To investigate the role of antigen specificity in PD-1 induction, transgenic mice with T cell receptors specific for non-murine ovalbumin peptide (OT-1TCR) were inoculated with ALL; impressively, the significant induction of PD-1 and the dysfunctional degranulation was abolished without TCR recognition of the leukemia. Currently, we are evaluating the translatability of our murine observations in clinical patient samples and the therapeutic potentials of blocking the PD-1 axis in the setting of ALL. Given the pervasiveness of PD-1 and evidence for T cell dysfunction with ALL progression, elucidating the mechanism by which this process occurs will provide therapeutic targets for advancing ALL immunotherapy.

#41

A CD19-Specific Chimeric Antigen Receptor Endows CD4+ T Cells with Unexpected Anti-tumor Activity Mediated by Both Perforin/Granzyme and Death Receptor Mechanisms

Tasha L. Lin[1], Daniel W. Lee[1], James N. Kochenderfer[2], and Crystal L. Mackall[1]

[1]Pediatric Oncology Branch, CCR, NCI; [2]Experimental Transplantation and Immunology Branch, CCR, NCI

Adoptive immunotherapy with T cells genetically engineered to express chimeric antigen receptors (CARs) has shown early promising results in clinical trials for the treatment of cancer. However, factors resulting in durable antitumor effects require further investigation. We have been studying a CAR consisting of the single-chain variable fragment specific for CD19, TCR zeta-chain, and the CD28 co-stimulatory domain, against pre-B acute lymphocytic leukemia (ALL). Immunomagnetically enriched CD8+ and CD4+ CD19-CAR T cells were generated separately by retroviral transduction of anti-CD3/CD28 bead-activated T cells in the presence of IL-2. In 4-hour chromium-51 release assays, CD8+ CARs demonstrated potent killing (specific lysis 80-100%) against NALM6, a human ALL cell line, while CD4+ CARs mediated significantly reduced killing. CAR T cells were administered to immunodeficient NOG mice engrafted with NALM6-GL (NALM6 stably expressing GFP and firefly luciferase). On bioluminescent imaging, mice receiving equivalent numbers of CD8+ or CD4+ CAR T cells eliminated NALM6-GL within 2 and 4 days, respectively. This demonstrates slower but surprising CD4+ CAR-mediated killing that is not captured by short-term in vitro assays. In 8-hour chromium-51 release assays, CD4+ CARs demonstrate similar cytotoxicity to that of CD8+ CARs at 4 hours. To elucidate the mechanism of CD4+ CAR cytotoxicity, assays were repeated in the presence of chloroquine, an inhibitor of perforin-granzyme-dependent cytotoxicity, or neutralizing antibodies to FasL and TRAIL. At 8 hours, studies indicate that the perforin-granzyme and Fas-FasL death receptor pathways contribute to both CD8+ and CD4+ cytotoxicity, but that the latter has a greater contribution in CD4+ CARs than in CD8+ CARs. TRAIL did not contribute to cytotoxicity in either subset. We report unexpected cytolytic activity in vitro and in vivo by CD4+ CD19 CAR T cells against ALL that occurs more slowly than with CD8+ CAR T cells but with similar mechanisms of cytotoxicity.

#42

Replacing gc-cytokine requirement with the serine/threonine kinase Pim1 during ab T lineage cell development

Brett A. Linowes[1], Anna S. Nam[1,2,3], Davinna L. Ligons[1], Changwan Hong[1], Hilary R. Keller[1], Megan Luckey[1], and Jung-Hyun Park[1]

[1]Exp. Immunol. Branch, NCI, NIH, Bethesda, MD; [2]University of Missouri School of Medicine, Columbia, MO; [3]Howard Hughes Medical Institute, Bethesda, MD

Signaling over the cytokine receptor gc is a critical and non-redundant requirement for the development of all lymphoid lineage cells. In mice, absent gc cytokine signaling results in a complete block of all B- and T-cell development and also in the failure to generate gd T cells, NKT cells and TCRb+CD8aa intraepithelial lymphocytes (IEL). The reasons for such gc cytokine requirement are manifold. Among others, induction of the serine/threonine kinase Pim-1 is a major requirement as Pim1 provides survival and increased metabolic activity downstream of gc cytokines. As such, we asked whether Pim1 is sufficient to restore lymphocyte development in the absence of gc cytokines. By analyzing gc-deficient mice transgenic for Pim1, here we show that Pim-1 significantly restores ab T cell development in the thymus and greatly improves peripheral homeostasis of mature T cells. Strikingly, Pim1 failed to rescue any other lymphoid lineage cells except for ab T lineage cells, as we still observed a complete lack of B-, gd T- , NKT-cells and TCRb+CD8aa IELs. Thus, these results uncover distinct requirements of gc downstream signaling between ab T-cells and all other lymphoid lineage cells, and propose Pim-1 as a novel effector molecule, sufficient to drive ab T cell development and differentiation in the absence of gc cytokines.

#43

Formylpeptide receptors play a pivotal role in host defense against Listeria monocytogenes

Mingyong Liu[1,2], Keqiang Chen[2], Teizo Yoshimura[2], Ying Liu[2], Wanghua Gong[3], Aimin Wang[4], Ji-Liang Gao[5], Philip W. Murphy[5], and Ji Ming Wang[2]*

[1]Department of Spine Surgery, Daping Hospital, Third Military Medical University, Chongqing, China; [2]LMI, CIP, CCR, NCI-Frederick; [3]Basic Research Program, SAIC-Frederick, Inc.; [4]Center for Orthopedics, Daping Hospital, Third Military Medical University, Chongqing, China; [5]Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, MD

Innate immune responses mediated by phagocytic leukocytes, such as neutrophils and monocytes, are the first line of host defense against microbial infection. Phagocytes accumulate at the site of infection presumably by migration in response to bacterial chemotactic products. In this study, we examined the contribution of G-protein coupled formylpeptide receptors (FPRs) to the host resistance to *Listeria monocytogenes* infection. We found that the phagocytic leukocytes from mice deficient in either mFPR1 or mFPR2 exhibited reduced chemotaxis response to Listerial lysate and its peptide fragment. Deletion of both mFPR1 and mFPR2 completely abrogated mouse phagocyte chemotaxis induced by *Listerial* products. In vivo, while mFPR1 or mFPR2 single deficient mice each showed decreased survival after *Listerial* infection, mice lacking both receptors markedly increased the susceptibility to infection in association with enhanced bacterial load in the liver and spleen. We further found that phagocyte accumulation in the liver and spleen as well as the phagocyte production of superoxide was severely impaired in mFPR deficient mice infected with *Listeria*. Our results demonstrate that FPRs are critical for host defense against *Listerial* infection.

#44

Inhibition of STAT1 signaling abrogates GVHD through a tolerogenic plasmacytoid dendritic cell

Nicole Nasholm[1], Christian Capitini[2], and Terry Fry[1]

[1]Pediatric Oncology Branch, CCR, NCI, Bethesda, MD; [2]Pediatric Hematology/Oncology, University of Wisconsin, Madison, WI

Allogeneic bone marrow transplantation (BMT) is limited in use and efficacy by the occurrence of graft versus host disease (GVHD), in which donor T cells in the bone marrow graft become activated and target host tissues. We have previously shown that in a murine model of acute GVHD, transplantation of MHC-matched mHA-mismatched bone marrow deficient in signaling through the IFNg receptor 1 (IFNgR1) leads to diminished GVHD (B6->B6 x C3H.SW) and sustains anti-tumor effects after a delayed lymphocyte infusion (DLI). Furthermore, when recipients are deficient in STAT1, a downstream transcription factor of IFNgR1, they also are protected from GVHD (129->C3H.SW) with an accompanied significant increase in plasmacytoid dendritic cells (pDCs). Research surrounding pDCs has suggested that these cells can be both tolerogenic and stimulatory in different conditions. In this study we sought to characterize the pDCs in our model on day 14 post-BMT at the time of the DLI. STAT1 deficient pDCs showed a higher degree of tolerogenic phenotypes as determined by cytokine production, gene expression and cell surface markers. Additionally, to determine the importance of STAT1 deficient pDCs in GVHD resistance, we treated mice receiving STAT1 deficient bone marrow (129->C3H.SW) with a PDCA antibody around the time of the DLI. Mice that received the pDC depleting antibody showed worsened GVHD, thereby suggesting the importance of STAT1 deficient pDCs in GVHD resistance.

#45

Mutational Analysis Provides Insight Into The Protective Role Of KIR3DS1 In HIV Pathogenesis

Geraldine M. O'Connor[1], Bernard A.P. Lafont[2], Andrew G. Brooks[3], David Price[4], and Daniel W. McVicar[1]

[1]Cancer and Inflammation Program, CCR, NCI; [2]Non-Human Primate Immunogenetics and Cellular Immunology Unit, NIAID; [3]Department of Microbiology and Immunology, University of Melbourne; [4]Department of Infection, Immunity and Biochemistry, Cardiff University School of Medicine

The KIR family, which includes members that transduce both positive and negative signals, is important in the control of Natural Killer (NK) cells. KIR3DL1 inhibits NK cell activity upon engagement by its ligand HLA-Bw4. The highly homologous KIR3DS1 is an activating receptor, which has implicated in the outcome of a variety of pathological situations, including control of viral load and opportunistic infections in HIV. In spite of strong genetic evidence supporting a functional interaction between KIR3DS1 and HLA-Bw4, no direct interaction between these molecules has been detected. Relative to KIR3DL1, KIR3DS1 is characterized by the presence of three unique amino acids substitutions in positions known to be important in the interaction with HLA. We mutated these residues in KIR3DL1 to the corresponding residue in KIR3DS1 and analyzed the ability of the resultant receptor to bind pHLA-B*5701-LF9. One mutation in particular, Leu166Arg, resulted in a dramatic loss of binding, suggesting that this substitution may be primarily responsible for the lack of broad reactivity of KIR3DS1 with HLA-Bw4. As KIR receptors are known to interact with the presented peptide at P8, we next analyzed the ability of 3DL1 Leu166Arg to bind to a panel of B*5701-LF9 tetramers with substitutions at P8. We found that 3DL1 Leu166Arg bound specifically to B*5701-LF9 with phenylalanine at P8, and this reactivity was also seen with KIR3DS1. Based on this reactivity, and the known peptide binding profile of B*5701, BLAST analysis revealed the presence of a HIV-derived peptide that we predict will bind to B*5701 and allow recognition by KIR3DS1. These finding suggests a model in which KIR3DS1 does not typically recognize HLA-B*5701, but changes in the presented peptide during e.g. HIV infection permit KIR3DS1 binding. Engagement of KIR3DS1 and subsequent NK cell activation would then be predicted to help control viral replication and disease outcome.

#46

Prostate cancer-infiltrating mast cells suppress anti-tumor immunity through a TGF-beta- and IL-13-dependent mechanism

Stephanie K. Watkins[1], Katherine E.R. Stagliano[1], Jonathan M. Weiss[2], Alasdair M. Gilfillan[3], Stefan Ambs[4] and Arthur A. Hurwitz[1]

[1]Tumor Immunity and Tolerance Section, Laboratory of Molecular Immunoregulation, and [2]Experimetal Therapeutics Section, Laboratory of Experimental Immunology, Cancer and Inflammation Program, NCI, Frederick, MD; [3]Mast Cell Biology Section, Laboratory of Allergic Disease, National Institute of Allergy and Infectious Diseases; [4]Laboratory of Human Carcinogenesis, CCR, NCI, NIH, Bethesda, MD

Immune suppression is a major obstacle in the success of cancer immunotherapy and may involve suppressive T cells and tolerogenic tumor-associated dendritic cells (DCs). We now identify a mechanism by which tumor-associated mast cells (TA-MCs) exert immune suppressive activity on T cells and DCs. TA-MCs were found to suppress Ag-specific T cell responses and secrete IL-13 and TGF-beta which induced tolerogenic DCs during in vitro co-culture. Blocking IL-13 and TGF-beta in vivo prevented induction of DC tolerogenicity and resulted in reduced tumor burden. Analysis of human tumor tissue samples revealed infiltration of MC (CD117+/FceR+) at a frequency of 20% of the leukocytes, a sub-population of which were found to express IL-13 and suppress PBMCs upon in vitro culture. Further, in vitro treatment with IL-13 and TGF-beta induced human monocyte-derived DC to become tolerogenic. Our findings reveal novel mast cell-mediated mechanisms that favor immune tolerance over tumor immunity and therefore provide promising targets for the enhancement of cancer immunotherapy.

Immune suppression is a major obstacle in the success of cancer immunotherapy and may involve suppressive T cells and tolerogenic tumor-associated dendritic cells (DCs). We now identify a mechanism by which tumor-associated mast cells (TA-MCs) exert immune suppressive activity on T cells and DCs. TA-MCs were found to suppress Ag-specific T cell responses and secrete IL-13 and TGF-beta which induced tolerogenic DCs during in vitro co-culture. Blocking IL-13 and TGF-beta in vivo prevented induction of DC tolerogenicity and resulted in reduced tumor burden. Analysis of human tumor tissue samples revealed infiltration of MC (CD117+/FceR+) at a frequency of 20% of the leukocytes, a sub-population of which were found to express IL-13 and suppress PBMCs upon in vitro culture. Further, in vitro treatment with IL-13 and TGF-beta induced human monocyte-derived DC to become tolerogenic. Our findings reveal novel mast cell-mediated mechanisms that favor immune tolerance over tumor immunity and therefore provide promising targets for the enhancement of cancer immunotherapy.

#47

Alarmin HMGN1 Boosts Antitumor Immunity

Feng Wei[1], De Yang[2], Poonam Tewary[1], Xin Chen2, O. M. Zack Howard[1], and Joost J. Oppenheim[1]

[1]NCI/CCR/LMI; [2]SAIC-Frederick, Basic Research Program, NCI-Frederick, Frederick MD

Alarmins are endogenous mediators that rapidly become available in peripheral tissues in response to danger signals and are capable of enhancing the induction of innate and adaptive immune responses by promoting the recruitment and maturation of antigen presenting cells (APCs). We have previously shown that high-mobility group nucleosome-binding protein 1 (HMGN1) possesses the properties of an alarmin. In this study, we expressed and purified HMGN1 from an insect cell expression system (riHMGN1). This riHMGN1 demonstrated alarmin activities including in vivo recruitment of APCs and in vitro activation of APCs. In a classic antigen-specific proliferation scheme, ovalbumin (OVA) combined with riHMGN1 promoted greater OVA-specific T cell proliferation than OVA alone or OVA combined with alum. We also observed that EG7 tumor, a mouse thymoma transfected to overexpress OVA, grew faster in Hmgn1-/- mice than in wild type mice. Furthermore, EG7-bearing Hmgn1-/- mice had fewer splenic OVA-specific CD8 cells, suggesting that endogenous HMGN1 is required to develop immune responses. On the other hand, when established in wild type mice, EG7-N1, a HMGN1-expressing EG7 tumor cell line, grew slower than control EG7 cell line cells, while both cell lines proliferated equally in vitro, suggesting that the murine immune system was awakened by the HMGN1 expressed by the tumor. These results support the hypothesis that HMGN1 contributes to the development of antitumor immunity.

#48

Humanizing high affinity monoclonal antibody against GPC3 for liver cancer therapy

Yifan Zhang, Yen Phung, Wei Gao, Mitchell Ho

Laboratory of Molecular Biology, CCR, NCI

Humanizing high affinity monoclonal antibody against GPC3 is an attractive target in the treatment of hepatocellular carcinoma. Our lab has developed several anti-GPC3 antibodies in mice which have higher affinity to the target than that of the current antibody. In the proposed study, we will convert these antibodies into therapeutic forms. In one direction, we will humanize it. In the other direction, we will make the immunotoxin ScFv-peudomonas exotoxin fusion protein. We will evaluate them in vitro and in vivo. This work will potentially lead to the development of a new antibody drug.

Molecular and Cell Biology, Virology, and Bioinformatics II

Oral Presentation

Chronic myeloid leukemia (CML) exosomes promote angiogenesis in a Src-dependent fashion in vitro and in vivo

Marco Mineo[1], Simona Taverna[2], Anna Flugy[2], Giacomo De Leo[2], Riccardo Alessandro[2] and Elise C. Kohn[1]

[1]Molecular Signaling Section, Medical Oncology Branch, CCR, NCI, Bethesda, MD; [2]Dipartimento di Biopatologia e Biotecnologie Mediche e Forensi, Sezione di Biologia e Genetica, Universit di Palermo, Palermo, Italy

CML is an uncontrolled proliferation of bone marrow myeloid cells driven by the constitutively active fusion product tyrosine kinase BCR/ABL. Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is newly recognized as a factor in CML progression. Exosomes are microvesicles that play an important role in cell-to-cell communication both in physiological and pathological conditions. The role of exosomes released by CML cells in angiogenesis is emerging; however, little is known about the mechanisms involved in this process. We isolated and characterized exosomes released by K562 CML cells and we demonstrated their ability to stimulate human vascular endothelial cells (HUVECs) tube differentiation on Matrigel. K562 exosomes induced an increase of the cumulative tube length in a dose-dependent manner, with a maximum effect at 10µg/ml ($p=0.003$). Next, we evaluated the effect on exosome behavior of imatinib and dasatinib, two tyrosine kinase inhibitors in use in CML treatment. K562 CML cell treatment with either imatinib or dasatinib reduced exosome release by 58% and 56%, respectively ($p<0.01$). Dasatinib treatment of HUVECs strongly reduced exosome-induced vascular differentiation ($p=0.0002$). On the contrary, little effect was observed following treatment with imatinib. Vascularization of an exosome containing Matrigel plug in vivo was markedly inhibited by oral administration of dasatinib ($p<0.01$), but not imatinib. Immunofluorescence analysis showed increased exosome-induced Src and FAK phosphorylation in HUVECs. Both FAK and Src phosphorylation were increased at points of membrane-matrix contact. Immunoblot analysis confirmed that K562 exosomes induced a dasatinib-sensitive phosphorylation of Src and FAK and their downstream effectors, Erk and Akt. Again, imatinib was minimally active against exosome stimulation of HUVEC cell signaling. Thus, K562 CML exosomes stimulate angiogenesis in vitro and in vivo in a dasatinib-sensitive fashion. This credentials exosomes and angiogenesis as molecular targets in CML via activation of Src both in leukemia and its microenvironment.

Oral Presentation

ADP-Ribosylation Factor 1 in HIV-1 Assembly and Release

Philip R. Tedbury and Eric O. Freed

Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI, Frederick, MD

HIV-1 budding is driven by the Gag polyprotein precursor and requires both homotypic interactions between Gag molecules and interactions between Gag and host cell components. Work in this lab has previously described ADP ribosylation factors (Arfs) as host factors affecting the release of HIV-1. Dominant active Arf6 inhibits release by relocalizing phosphatidylinositol-4,5-bisphosphate (PIP2), the trigger for membrane binding by HIV-1 Gag, to intracellular vesicles leading to a loss of Gag at the plasma membrane. To elucidate the role of other members of the Arf family we created a panel of Arf expression constructs including wild-type, dominant-negative (DN), dominant-active and unmyristylated mutants. These were transfected into HeLa cells with HIV-1 provirus and virus release assayed. Cotransfection of a luciferase expression vector was performed to control for non-specific toxicity. The data obtained revealed that only Arf1 DN inhibited virus release without affecting cell viability. Further experiments revealed that Arf1 DN induced processing defects in Gag, consistent with loss of membrane binding. A mechanism mediated via PIP2 perturbation was excluded by examining localization when co-expressed with Arf1 DN: PIP2 remained at the plasma membrane. To define the mechanism by which Arf1 DN interferes with HIV-1 assembly, we are taking advantage of the fact that the closely related protein Arf3 is inactive against HIV-1; Arf1 and 3 differ by only 7 amino acids, 3 located in the N-terminal alpha helix and 4 in the C-terminal helix. These alpha helices determine the stability of the membrane interaction and the localization of the Arf, respectively. Chimeras of Arf1 DN and Arf3 DN are being used to examine the properties of Arf1 DN that intersect with Gag trafficking. These approaches are revealing novel insights into the interactions between HIV-1 and the host cell, Gag trafficking and the distinct cellular functions of Arf proteins.

Oral Presentation

Protein SUMOylation is synthetic lethal to oncogenic KRAS in colorectal cancer cells

Bing Yu[1], Steve Swatkoski[2], Mengtzu Weng[1], Dennis Hsu[1], Changwoo Lee[1], Marjan Gucek[2], Ji Luo[1]

[1]Molecular Oncology Branch, CCR, NCI; [2]Proteomics Core Facility, NHLBI

Oncogenic Ras mutations are found in ~30% human cancers. This is a subset of cancers often associated with poor prognosis and yet without any effective targeted therapy. To date, Ras itself is pharmacologically intractable and inhibitors against major Ras downstream effectors have not been proved effective in clinical trials. Thus, to better characterize the genetic dependencies of Ras-driven tumors, we conducted a genome-wide RNAi synthetic lethal screen against the Ras oncogene and unexpectedly identified SUMO ligases in this screen. We found that blocking protein SUMOylation by knocking down SUMO E1 ligase SAE1 or E2 ligase Ubc9 with shRNAs modestly inhibited growth, but dramatically blocked colony formation of KRAS mutant cells rather than wild type cells in KRAS isogenic cell models and a panel of colorectal cancer cell lines. Meanwhile, SUMO inhibition completely depleted anchorage-independent growth of inducible KRAS-transformed cancer cell. These effects could be rescued by compensation of wild-type Ubc9 rather than SUMOylation defective mutant, demonstrating SUMO-conjugating function of Ubc9 is critical for KRAS mutant cells. A further siRNA screen focused on SUMO pathway genes indicated KRAS mutant cells depend more on PIAS1 and SUMO2 rather than SUMO1. To further address the functional roles of SUMOylation in KRAS mutant cells, we compared SUMOylation profiles between KRAS mutant and wild type cells with quantitative proteomics and found 40 differentially SUMOylated proteins. This KRAS-related SUMOylation signature contains proteins regulating gene expression and cellular signaling. Overall, these results indicated a critical contribution of protein SUMOylation in sustaining KRAS mutant cancer cells. So far, this study revealed the functional role of SUMOylation in KRAS-driven cancers and offered potential therapeutics for Ras mutant tumors by targeting their non-oncogene addiction.

Oral Presentation

Functional Redundancy in HIV-1 Assembly

Ina P. O'Carroll[1], Rachael M. Crist[1], Jane Mirro[1], Demetria Harvin[1], Ferri Soheilian[2], Anne Kamata[2], Kunio Nagashima[2], and Alan Rein[1]

[1]HIV Drug Resistance Program, CCR, NCI; [2]Advanced Technology Program, SAIC-Frederick, Inc., Frederick, MD

The human immunodeficiency virus type I (HIV-1) affects millions of people worldwide. The ability of the virus to develop resistance to available treatments necessitates the search for new ways to battle HIV-1. Assembly of the newly synthesized viral particle is a potential therapeutic target and, therefore, a better understanding of the process is necessary. In mammalian cells, assembly of immature viruses can be achieved by solely expressing Gag, one of the structural polyproteins encoded by the viral genome. Gag consists of three subdomains: 1) the matrix (MA), which binds the plasma membrane mainly via a myristate moiety; 2) the capsid (CA), in which a dimerization interface has been identified; and 3) the nucleocapsid (NC), which is the main genomic RNA-binding domain. RNA (or any nucleic acid) is required for Gag molecules to assemble in vitro. However, in vivo, deletion of NC in HIV-1 does not affect the ability of Gag to form virus-like particles. In addition, treatment of isolated immature particles with RNase does not disrupt them, suggesting that RNA is not needed for the maintenance of the virus structure. We have investigated the role of RNA in assembly in vivo by analyzing the content of DelNC HIV particles. We found that very little total RNA is present (~10% compared to wild type). To understand how HIV-1 assembles with very little dependence on RNA, we designed several mutants that disrupt i) the Gag dimer interface on the CA domain, ii) Gag-membrane interactions, iii) NC-RNA interactions, and iv) double mutants combining the three types of interactions. We find that disruption of any one type of interaction is not sufficient to disable assembly. However, combination of any two mutants completely abolishes or significantly affects assembly. Thus, in vivo, HIV-1 assembles with some functional redundancy that renders the presence of RNA obsolete.

Oral Presentation

Lysophosphatidic acid signaling regulates primary cilia assembly

Vijay Walia, Jeffery Gray, Yan Pang, and Christopher J. Westlake

Laboratory of Cell and Developmental Signaling, NCI-Frederick

Defects in primary cilium formation and signaling are associated with a growing list of genetic diseases and have been linked to certain cancers. Primary cilia are regulated by the cell cycle, dissembled prior to mitosis and reassembled in interphase or Go cells. Typically, cultured cells develop cilia only following serum starvation. To identify constituents present in serum that regulate ciliogenesis, we screened 30 growth factors ubiquitously present in human serum and identified lysophosphatidic acid (LPA) as a novel regulator of ciliogenesis. LPA binds to the LPA receptor (LPAR), a family of G-protein coupled receptors. Using RNAi and inhibitor studies we show that LPAR1 specifically regulates LPA-dependent signaling to block ciliogenesis in immortalized human retinal pigment epithelial (RPE) cells. Next we investigated downstream cell signaling cascades activated by LPA-LPAR1 using kinase inhibitors associated with the LPA pathway. We find that PI3k-Akt signaling strongly modulates cilia growth. Using live microscopy, we show that blockage of LPA signaling and PI3k or Akt kinase activity promotes Rab11-dependent preciliary membrane transport required to build the primary cilium membrane. Interestingly, GFP-LPAR1 is detected on the plasma membrane and Rab11-positive intracellular vesicles in RPE cells in the presence of serum, but relocalizes completely to the plasma membrane within minutes of serum depravation, suggesting LPA-AKT signaling may directly modulate Rab11 ciliary transport. These findings demonstrate a novel LPA-Akt signaling network in the regulation of ciliogenesis.

#49

Shaping the Developing Primary Cilium Membrane

Quanlong Lu and Christopher J Westlake

Laboratory of Cell and Developmental Signaling, CCR, NCI-Frederick

Primary cilium formation or ciliogenesis involves coordinated assembly of a microtubule-based axoneme from the mother centriole and vesicular membrane fusion that forms a ciliary membrane around the axoneme. Ciliary membrane assembly is initiated by the Rab11-endosome recycling compartment which delivers the Rab8 guanine nucleotide exchange factor (GEF), Rabin8, to the centrosome to activate Rab8 to form the ciliary membrane. During cilia formation, reorganization of the developing ciliary membrane is thought to occur as the ciliary vesicle is reshaped into a tubule structure around the developing axoneme. In live spinning disk confocal imaging studies using fluorescent protein fusions of Rab8 and Centrin1, a centrosomal marker, we could monitor primary cilium membrane assembly. To further investigate the membrane structural rearrangements occurring during the formation of the primary cilium, we utilized correlative light and electron microscopy (CLEM) to visualize events leading to ciliary vesicle formation, ciliary membrane and axoneme elongation, fusion with the plasma membrane and the development of the ciliary pocket. Next we investigated the potential mechanisms involved in these ciliogenesis processes by examining the potential role of EHD proteins, a family of membrane curving proteins known to bind to Rab11 and Rab8 effector proteins. EHD1 and EHD3, but not EHD2 and EHD4, localized to Rabin8 centrosomal vesicles prior to ciliogenesis and were present on the developing primary cilium membrane. While EHD proteins are dispensable for Rabin8 centrosomal transport, EHD1, but not EHD3, is required for RPE cell ciliogenesis based on RNAi studies. EHD1 -/- MEFs also failed to form normal cilia, but could be rescued by human EHD1 or EHD3 expression. Together these results suggest that EHD1 and EHD3 function in shaping the developing ciliary membrane. Interestingly, in mature cilia, EHD1 and EHD3 localizes dynamically to the proximal region of cilia. Using CLEM, super resolution spectral illumination microscopy (SIM), and total internal reflection fluorescence (TIRF) microscopy, we show that EHD1 localizes to the ciliary pocket, a poorly characterized membrane domain separating the ciliary membrane from the plasma membrane. TIRF imaging indicates EHD1 is dynamically localized to ring-like structures around the ciliary pocket and to membrane tubules extending from this membrane domain. We are investigating whether EHD proteins function in trafficking to/from the ciliary pocket and in maintenance of the ciliary pocket structure.

#50

Detection of LacNAc and GlcNAc moieties on the cells surface with Glycosyltransferases

Natalia Mercer[1], Boopathy Ramakrishnan[1,2], Marta Pasek[1], Elizabeth Boeggeman[1,2], Luke Verdi[1], and Pradman K. Qasba[1]

[1]Structural Glycobiology Section, CCR-Nanobiology Program, CCR, NCI-Frederick; [2]Basic Science Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD

In the present work, we have used mutant galactosyltransferases previously developed in our lab to detect GlcNAc and LacNAc on the surface of human cervical cancer cells (HeLa). The mutant enzymes have a cavity that has been carved in the donor site to accommodate UDP-Gal with a chemical handle at C2, such as azide (GalNAz) or keto group (C2-keto-Gal). The chemical handles are used for conjugation with fluoroprobes or biotin carrying bioorthogonal group to detect the acceptor GlcNAc or LacNAc. A double mutant of β1,4-galactosyltransferase (β1,4GalT), Y289L-M344H, is used for detecting GlcNAc residue. The Tyr289Leu (Y289L) mutation allows the carving of the cavity to accommodate UDP-GalNAz, whereas the second mutation, Met344His (M344H), located in the enzymes metal binding site, changes the metal cofactor requirement from Mn2+ to Mg2+. Detection was investigated using confocal microscopy and flow cytometry. Green membrane fluorescent signal (corresponding to DIBO-Alexa 488) was detected on the HeLa cells only when cells were pre-treated with sialidase and galactosidase enzymes, indicating that glycans with free GlcNAc residues are not abundant on the surface of HeLa cells. The LacNAc moiety on the cell surface was detected using α1,3-galactosyltransferase (α1,3GalT) mutant enzyme, α1,3GalT-280AGG282 , which transfers GalNAz or C2-keto-Gal to N-acetyl-lactosamine (LacNAc). The GalNAz or C2-keto-Gal labeled glycans were coupled with alkyne- or aminooxy-biotin, respectively. On fixed cells, coupled biotin was detected with streptavidin-Alexa 488. On extracts, coupled biotin was detected with streptavidin-HRP. Fluorescent signal could be detected on cell membranes, as opposed to control (no UDP-GalNAz). Cells that were pre-treated with sialidase, increased the signal intensity, indicating that the density of exposed LacNAc residues was augmented by the removal of sialic acid. Similar results were obtained by Western Blot. In conclusion, the use of β1,4GalT-Y289L-M344H and α1,3GalT-280AGG282 enzymes could be a powerful tool to study the cells glycophenotype.

#51

PARP inhibitors poison PARP1 and PARP2 by stabilizing PARP1-DNA and PARP2-DNA complex

Junko Murai[1], Shar-yin N. Huang[1], Amelie Renaud[1], Shunichi Takeda[2], and Yves Pommier[1]

[1]Laboratory of Molecular Pharmacology, CCR, NCI, NIH; [2]Kyoto University, Japan

Poly(ADP-ribose) (PAR) polymerase (PARP) inhibitors are in clinical trials with topoisomerase I inhibitors and alkylating agents, and in patients with BRCA1- or BRCA2-deficient tumors. PARP inhibitors were initially proposed to prevent the repair of single strand breaks (SSBs) by inhibiting the catalytic activities of PARP1 and PARP2. However, recent studies propose that PARP1 is trapped on DNA by PARP inhibitors, and the trapped PARP1-DNA complexes can be toxic lesions by interfering with DNA replication, while direct genetic evidence to support this model has been lacking. We show that PARP1-/- chicken DT40 cells and PARP1 knockdown human DU145 cells are resistant to olaparib (a leading PARP inhibitor in clinical development) compared to their parent cells, indicating that the presence of PARP1 contributes the cytotoxicity of olaparib. We also find that PARP1 accumulates in chromatin fractions after olaparib treatment. PARP1 accumulation is greatly enhanced by combining olaparib with the alkylating agent, MMS. Considering that PARP1 has zinc finger DNA binding domains and binds to damaged DNA sites, we assumed that olaparib stabilized PARP1-DNA complexes on damaged DNA sites. Although PARP1-/- DT40 cells were hypersensitive to MMS, very low concentrations of MMS with olaparib produced a much greater cytotoxicity in wild type cells than in PARP1-/- cells. These results indicate that olaparib-induced PARP1-DNA complexes are more cytotoxic than the unrepaired SSBs resulting from the absence of PARP1. PARP2-DNA complexes were also stabilized by olaparib, and had cytotoxic potency. We tested another leading PARP inhibitor, veriparib, which induces PARP inhibition as well as olaparib, and found that the potency to poison PARP1 by veriparib was much less than that by olaparib. Our study provides new insights for the molecular and system pharmacology of PARP inhibitors and their application for cancer therapy.

#52

The Kaposis sarcoma-associated herpesvirus ORF57 is not a bona vide export factor

Guy R. Pilkington[1], Vladimir Majerciak[2], Jenifer Bear[1], Zhi-Ming Zheng[2] and Barbara K. Felber[1]

[1]Human Retrovirus Pathogenesis Section, Vaccine Branch, CCR, NCI-Frederick; [2]Tumor Virus RNA Biology Section, HIV and AIDS Malignancy Branch, CCR, NCI, Bethesda

Kaposis sarcoma-associated herpesvirus (KSHV) ORF57 is a viral early protein essential for virus replication, which is implicated in a number of posttranscriptional regulatory events, such as splicing, RNA stabilization, export and translation. Predominantly a nuclear protein, ORF57 interacts with RBM15 and OTT3, which are cellular cofactors to the nuclear export receptor NXF1, thereby providing a mechanism for the export of viral RNA. Recent data suggests that ORF57 recruits the TREX complex to viral RNA, and that ORF57 is directly involved in the nuclear export. In this study, we show that while ORF57 promotes expression of a selection of KSHV viral intronless RNAs, there is no evidence that it is directly involved in nuclear export of these transcripts. Utilizing two independent RNA export assays, we demonstrate that ORF57 is not a bona fide export factor. KSHV ORF57 has functional homologs in all members of the mammalian herpes viruses, including ICP27 in herpes simplex virus-1 (HSV-1), EB2 in Epstein-Barr virus (EBV) and UL69 in human cytomegalovirus (HCMV). These proteins were also tested in the RNA export assay and, similar to KSHV ORF57, none of them showed any specific export activity, supporting the model that export function is not within the repertoire of these multifunctional proteins. Even though we have shown that ORF57 is not an export factor, ORF57 is still critical for the posttranscriptional regulation of many KSHV genes and remains essential for the survival of the virus.

#53

Glioma cell density affects sensitivity to receptor tyrosine kinase inhibitor therapy in vitro

Andrew L. Smith and Jayne M. Stommel

Radiation Oncology Branch, CCR, NCI

Receptor tyrosine kinases (RTKs) are an attractive therapeutic target for multiple cancers, including glioblastoma multiforme. Glioma cells lines typically express multiple activated receptor tyrosine kinases, and simultaneous targeting of these active kinases reduces tumorgenicity and promotes cell death in vitro. Physical cues such as adhesion can affect RTK activity and downstream signaling. In particular, RTKs have been shown to localize to cell-cell contacts and form complexes with adhesion molecules such as cadherins and integrins. We have previously shown that simultaneous pharmacological inhibition of EGFR, PDGFR, and c-MET using Tarceva, Gleevec, and PHA66572, respectively (collectively called TGP), induced dramatic cytotoxicity in multiple glioma cell lines after 72 hours of treatment. We hypothesized that sensitivity to these (and other) RTK inhibitors is influenced by cell adhesion and cell density. At low cell density, TGP treatment was highly toxic to LN18, LN382, and U87 glioma cells. However, at high density, toxicity was significantly reduced. Biochemical analysis indicated that the drugs efficiently inhibited EGFR, PDGFR, and c-MET phosphorylation and downstream signaling to AKT at both low and high density. No difference in cell-cycle status was observed between low and high density glioma cells over the course of the experiment. Studies employing a glioma cell-astrocyte co-culture system indicate that interaction between glioma cells, but not between glioma cells and normal astrocytes, promotes resistance to TGP-induced cytotoxicity. Furthermore, inhibition of cell-cell contact effectively sensitized high density cells to TGP. These data suggest that at high density, cell-cell contact between glioma cells may circumvent the need for RTK signaling; thereby limiting the efficacy of RTK mediated therapy. Ongoing studies are aimed at identifying the mechanism of resistance and finding agents that can restore sensitivity to RTK inhibition at high cell density.

#54

A Computational Mammalian Circadian Clock Model with Connections to the DNA Damage Response

Augustin Luna[1,2], Geoffrey McFadden[3], Mirit I. Aladjem[2] and Kurt W. Kohn[2]

[1]Laboratory of Molecular Pharmacology, NCI, Bethesda, MD; [2]Department of Bioinformatics, Boston University, Boston, MA; [3]Applied and Computational Mathematics Division, National Institute of Standards and Technology, Gaithersburg, MD

Circadian rhythms help synchronize daily changes in several processes including metabolism and DNA repair. Additionally, it is known that some tumor types suffer from considerably altered circadian rhythms. We present an extended computational model of a mammalian circadian clock that emphasizes the roles of chromatin remodeling and metabolic pathways on the regulation of circadian rhythms and the effects of DNA damage response on this regulation. Many mathematical circadian models focus on the core components and the role of phosphorylation as a key post-translational modification in circadian regulation; few models have explored other modifications. Recent evidence shows a role for NAD-dependent proteins in circadian control, including: SIRT1, a histone deacetylase, and PARP1, an ADP-ribosyltransferase. Also, there has been evidence that radiation induces phase advancements in the circadian rhythms of cells. We believe the regulation of NAD+ may be involved in these advancements, due to the dramatic activation of PARP1 resulting from DNA damage. To help understand the dynamics of the circadian regulation during DNA damage, we are developing a model focused on the regulation NAD+ levels and the activities of SIRT1 and PARP1. We have extended a basic mammalian model using methodology described in a Drosophila model to simulate histone acetylation. This has been expanded to include NAD+ levels via circadian-controlled NAMPT, a rate-limiting enzyme in NAD+ synthesis.

#55

A re-sequence analysis of genomic loci on chromosomes 1q32.1, 5p15.33 and 13q22.1 associated with pancreatic cancer risk

Hemang Parikh[1], Jinping Jia[1], Xijun Zhang[2,3], Charles C. Chung[1], Kevin B. Jacobs[2,3], Meredith Yeager[2,3], Joseph Boland[2,3], Amy Hutchinson[2,3], Laura Burdett[2,3], Jason Hoskins[1], Harvey A. Risch[4], Eric J. Jacobs[5], Rachael Z. Stolzenberg-Solomon[2], Stephen J. Chanock[1], Brian M. Wolpin[6,7], Gloria M. Petersen[8], Charles S. Fuchs[6,7], Patricia Hartge[2] and Laufey Amundadottir[1]

[1]Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD; [2]Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD; [3]Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [4]Yale University School of Public Health, School of Medicine, New Haven, CT; [5]Epidemiology Research Program, American Cancer Society, Atlanta, GA; [6]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; [7]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [8]Department of Health Sciences Research, Mayo Clinic, Rochester, MN

Genome-wide association studies (GWAS) have identified a set of genomic regions that harbor susceptibility alleles for pancreatic cancer risk. To fine-map regions identified by single nucleotide polymorphism (SNP) markers, we conducted a targeted re-sequencing analysis using Roche-454 across 428 kb in three pancreatic cancer susceptibility regions on chromosomes 1q32.1, 5p15.33 and 13q22.1, identified in European populations. An analytical pipeline for calling genotypes was developed using HapMap samples sequenced on chr5p15.33. The concordance rate between genotype calls from re-sequencing and 1000 Genome data (October 2011 release) for chr5p15.33 was 96.2% for HapMap CEU, 97.9% for HapMap CHB/JPT and 97.1% for HapMap YRI samples. The concordance between 1q32.1 and 13q22.1 and our previous pancreatic cancer GWAS genotype data was greater than 99%. Between 9.2 and 19.0% of variants called in the sequencing analysis were not present in 1000 Genomes for the respective continental populations. The majority of completely novel SNPs were less common (MAF ≤ 5%) or rare (MAF ≤ 2%), illustrating the value of enlarging the test sets for discovery of less common variants. Using the combined data set, we examined the haplotype blocks across each region using a tag SNP analysis (r2 > 0.8 for MAF ≥ 5%) in European populations and determined that at least 196, 243 and 63 SNPs are required for chr1q32.1, chr5p15.33, and chr13q22.1. Here, we have characterized germline variation in three regions associated with pancreatic cancer risk and show that targeted re-sequencing leads to the discovery of novel variants and improves the completeness of germline sequence variants for fine-mapping GWAS susceptibility loci.

#56

Fusion of p14arf to a Translocation Hotspot in a Patient with Melanoma, Deafness and DNA Repair Deficiency acts as a Negative Regulator of p14ARF and TBX1

Xiaohui Tan[1], Sarah Anzick[2], Sikandar G. Khan[1], Takahiro Ueda[1,3], Gary Stone[2], John J. DiGiovanna[1], Deborah Tamura[1], Daniel Wattendorf[4], David Busch, Carmen Brewer[5], Chris Zalewski[5], Robert Walker[2], John A. Butman[6], Andrew Griffith[5], Alexander Kovalchuk[8], Paul Meltzer[2], Paul Bergstresser[7] and Kenneth H. Kraemer[1]

[1]DNA Repair Section, Derm Br NCI; [2]Genetics Branch, NCI; [3]Pharm/Medical Device Agency, Tokyo, Japan; [4]Office of the Air Force Surgeon General; [5]Otolaryngology Br, NIDCD; [6]Radiology, CC; [7]Derm, Univ of Texas SW, Dallas, TX; [8]Laboratory of Genetics, NIAID

Translocation hotspot palindromic AT-rich repeat on chromosome 22 (PATRR22) in an unsequenced gap are still a riddle. We detected a novel a fusion of p14ARF to this region by studing a t(9;22)(p21;q11.2) translocation in a patient with melanoma, Digeorge Syndrom (DGS) /deafness. p14ARF was under-expressed and the DNA repair deficiency was corrected by transfection with p14ARF. Laser capture microdisseced melanoma revealed additional UVtype p14ARF mutations implicating p14ARF in the melanoma. Expression of TBX1 was reduced, however the 22q11.2 breakpoint was located 800kb away in PATRR22. The junction fragment allowed us to elongate PATRR22 surrounding sequence and suggsted PATRR22 is a part of intron within a new gene called PATRR22 Containing Translocation Hotspot (PCTH). The chimeric p14ARF-PCTH transcript acted as a negative regulator of p14ARF, TBX1and DNA repair capability. Our date not only demonstrats critical role of p14ARF and TBX1 in melanoma and DGS/deffness respectively but also explores a novel regulatory mechanism. The chimeric transcript could provide a unique target for skin cancer prevention in this patient.

#57

Crosslinking of subunits in ClpA hexamers reflects the high flexibility of the NBD2 domains

P. Dharkar[1], G.M. DeDonatis[2], and M.R. Maurizi[1]

[1]Laboratory of Cell Biology, NCI, NIH, Bethesda, MD; [2]Nanomedicine Development Center, University of Cincinnati, Cincinnati, OH

Escherichia coli ClpA, the regulatory component of the ATP-dependent ClpAP protease, consists of two ATP-binding regulatory domains, NBD1 & NBD2, that belong to the AAA superfamily. ClpA assembles into a hexamer in the presence of ATP. In the crystal structure of ClpA, NBD1 & NBD2 are connected by a short hinge. A proposed dynamic model of the ClpA hexamer describes the structure as transitioning between spiral and planar conformation due to movement around the hinge region. Electron microscopy has provided direct evidence of mobility within NBD2, reflecting swiveling of the large and small subdomains about a nucleotide-sensitive connector. The mobility of NBD1 and NBD2 about each other and the high mobility within NBD2 have made crystallization of ClpA hexamers difficult. We engineered cysteines into specific positions in an otherwise cysteineless ClpA and are using these sites to introduce crosslinks at strategic positions with the aim of reducing the range of motion in NBD2. Purified forms of two ClpA mutants, Met476Cys & Lys714Cys, have been assembled in the presence of ATP and crosslinked with bifunctional agents of varying lengths. Crosslinked species included the heterologous dimer predicted from the proximity of positions 476 and 714 in adjacent subunits of ClpA hexamers. However, we also obtained homologous species of dimers in which Met476 subunits were crosslinked to each other and Lys714 subunits were crosslinked to each other. Both types of dimers were dependent on assembly of ClpA hexamers, indicating a greater range of motion of NBD2 within assembled ClpA hexamers than was previously predicted. These results point to the prominent role mobility of NBD2 plays in ClpA activity and suggest that a dynamic interaction with ClpP might be needed to allow substrate translocation into the degradation chamber.

Translational, Clinical, and Epidemiology Research

Oral Presentation

Surprising Eradication of Pediatric ALL by CD4+ T cells with a CD19-Specific Chimeric Antigen Receptor

Daniel W. Lee[1], Tasha L. Lin[1], James N. Kochenderfer[2], Rimas J. Orentas[1], Elizabeth G. Gardner[1], and Crystal L. Mackall[1]

[1]Pediatric Oncology Branch, National Cancer Institute; [2]Experimental Transplantation and Immunology Branch, NCI

Pediatric acute lymphocytic leukemia (ALL) remains a difficult therapeutic challenge. Chimeric antigen receptors (CARs) are genetically engineered molecules that allow T cells to recognize and kill specific targets independent of the T-cell receptor (TCR). We are evaluating a CAR directed against CD19, present on nearly 100% of ALL blasts. Consisting of a CD19-specific single chain variable fragment, the TCRzeta signaling subunit and the co-stimulatory CD28 domain, CD19-CAR T cells (19CAR-T) are generated by retroviral transduction of activated T cells. Transduction efficiency averaged 50-80%. 19CAR-Ts specifically and robustly killed four CD19+ ALL but not CD19 target cell lines and produced significant levels of IFNγ, TNFα, and IL-2. In immunodeficient NOG xenografts, 19CAR-Ts eliminated engrafted NALM6-GL (ALL cell line stably expressing GFP and firefly luciferase) within 72 hours in 5/5 mice whereas all animals that received Mock cells required sacrifice within 18 days. Lower cell doses produced less potent antitumor effects and were associated with a lack of persistence of 19CAR-Ts. MHC-dependent CD4+ T cells are not classically cytotoxic. However, since CAR based recognition is MHC independent, we hypothesized that both CD4+ and CD8+ 19CAR-Ts may mediate cytotoxicity. So, these cells were isolated from the same donor, activated and transduced as before. In vitro assays demonstrated cytotoxicity from CD8+ 19CAR-Ts similar to unselected 19CAR-Ts but less cytotoxicity from CD4+ 19CAR-Ts. In xenografts, CD4+, 1:1 mix of CD8+:CD4+, and CD8+ 19CAR-Ts produced complete responses in 5/5 mice by Day 4. Leukemia clearance was rapid with CD8+ 19CAR-Ts but not durable as all mice relapsed. In contrast, CD4+ 19CAR-Ts cleared leukemia slower but responses were durable with CAR+ cells still detectable long-term. These results inform clinical development of this emerging therapy by emphasizing the importance of cell dose, cell persistence and the novel observation that CD4+ CAR-Ts may be critical effectors in mediating antitumor effects.

Oral Presentation

Dual PI3K/mTOR inhibition induces cell death in a defined subset of pancreatic cancer cell lines

Holger Pflicke[1], Yaroslav Teper[1], Joshua J. Waterfall[2], Paul S. Meltzer[2], Udo Rudloff[1]

[1]GI Oncology and HBP Section, Surgery Branch, CCR, NCI; [2]Genetics Branch, CCR, NCI

Pancreas cancer remains one of the deadliest cancers worldwide with a paltry 5-year survival rate of around 6%. In 2005 the receptor tyrosine kinase inhibitor erlotinib was approved by the FDA, improving the overall survival only marginally when compared to the then current standard of care (erlotinib plus gemcitabine vs gemcitabine alone: 6.24 vs 5.91 months). To improve receptor tyrosine kinase therapy in pancreas cancer we hypothesize that targeting downstream effector pathways as well as the establishment of molecular markers for appropriate patient selection will be key strategies. One pathway that is inappropriately activated in many cancers is the phosphatidylinositol 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway that regulates a diverse array of cellular activities most notably proliferation and survival. In this study we employ NVP-BEZ235, a dual PI3K/mTOR inhibitor that has shown promising preclinical activity in several cancers and is currently under clinical investigation in phase I/II trials for solid tumors, most notably breast cancer. We screened a large panel of pancreatic cancer cell lines with NVP-BEZ235 in standard growth inhibition assays and found induction of cell death at sub-micromolar concentrations in about 20% of the cell lines (NVP-BEZ235-sensitive) as shown by the determination of a lethal dose (LD50), Annexin V apoptosis assay as well as cell cycle analysis. Importantly, we also observed in vivo activity in NVP-BEZ235-sensitive cell lines in heterotopic xenografts when compared to NVP-BEZ235-resistant cell lines. Gene-expression analysis and subsequent validation of targets using available expression data of pancreatic cancer tissues allowed the establishment of a molecular signature defining sensitivity or resistance of pancreatic cancer cell lines to treatment with NVP-BEZ235. Selected targets are currently under further investigation. Our data indicate that targeting the PI3K-Akt-mTOR axis could be an effective strategy in certain subtypes of pancreas cancer.

Oral Presentation

CD81: A Novel, Specific and Highly Sensitive Marker in Flow Cytometric Diagnosis of Plasma Cell Dyscrasia

Prashant Tembhare[1], Constance Yuan[1], Neha Korde[2,3], Irina Maric[4], Katherine Calvo[4], Mary Ann Yancey[3], Ola Landgren[3], and Maryalice Stetler-Stevenson[1]

[1]Flow Cytometry Unit, Laboratory of Pathology, NCI; [2]Hematology Branch, National Heart, Lung, and Blood Institut; [3]Metabolism Branch, NCI; and [4]Hematology Section, Department of Laboratory Medicine, Clinical Center, NIH, Bethesda, MD

Background: The percent abnormal plasma cells (aPC) as determined by flow cytometry (FC) has been shown to be an independent risk factor for progression from myeloma precursor disease (monoclonal gammopathy of uncertain significance, MGUS; smoldering multiple myeloma, SMM) to multiple myeloma (MM). However, differentiation of aPCs from normal PCs (nPCs) in these patients is challenging. MM cell lines are know to underexpress the tetraspanin proteins (e.g. CD81, CD82) in comparison to nPCs. Although CD81, a nonglycosylated tetraspanin, is robustly expressed on the surface of nPCs, little information is available regarding its expression in the aPCs of MM, SMM and MGUS. We evaluate the expression of CD81 in conjunction with CD19, CD45 and CD56 in bone marrow (BM) aPCs and nPCs in MM, SMM and MGUS. Methods: BM aspirates from 41 patients (9 MGUS, 22 SMM, 7 MM, 3 non-neoplastic with clinical suspicion of MGUS) were analyzed with 8-color FC using multiple antibodies. The pattern of CD81 and other routinely used surface antigens and intracellular light chain expression by aPC & nPC was studied. Results: CD81 was strongly expressed by nPC (average mean fluorescent intensity (MFI): 11500) in contrast to aPC with abnormally weak expression (average MFI: 1487). CD81 was a highly reliable marker for the detection of clonal PC; with 90% sensitivity and 100% specificity. Evaluation of the combined pattern of expression of CD19 and CD81 resulted in 100% sensitivity and 100% specificity for detection of aPC. Conclusions: CD81 is a highly reliable marker in the detection of abnormal plasma cells in MM, SMM and MGUS. The combined approach of CD19 and CD81 is superior to other conventional marker combinations (i.e. CD19, CD45, and CD56) in terms of detection of clonal plasma cells and may replace their use in the clinical evaluation of BM aspirates for plasma cell processes.

Oral Presentation

Deficiency of CD47 in the tumor microenvironment enhances tumor responses to ionizing radiation, preserves immunosurveillance, and protects normal tissue through an increase in autophagy

David R. Soto-Pantoja[1], Masaki Terabe[2], Lisa Ridnour[3], William DeGraff[3], Jay A. Berzofsky[2], and David D. Roberts[1]

[1]Laboratory of Pathology; [2]Molecular Immunogenetics and Vaccine Research Section; [3]Radiation Biology Branch, CCR, NCI, NIH, Bethesda, MD

Over one million new cancer cases will be diagnosed in the US this year and over half of these patients will require radiation (IR) therapy as part of their course of treatment, however radiotherapy is limited by damage to normal tissue, decreasing radiocurability. The urgent need to find novel strategies to kill cancer cells but leave healthy ones untouched was highlighted by the president in his State of the Union address. We previously demonstrated that therapeutic targeting of CD47 confers selective radioprotection to normal tissue. Conversely, blockade of CD47 enhances the radiation-induced growth delay in two mouse models. One possible explanation is that targeting CD47 protects immune cells to enhance tumor immune surveillance. To test this we used a mouse adoptive T-cell transfer model using 15-12 RM mouse fibrosarcoma tumors in athymic mice. Adoptive transfer of CD8+ T-cells in combination with CD47 blockade and IR resulted in a significant reduction in tumor growth when compared to tumors of mice irradiated in combination with the T cell transfer (185.7 49.7 vs. 369.7 123.3 respectively, N=8). Similarly, blockade of CD47 enhanced antigen-dependent CD8+-mediated cytotoxicity of tumor cells in vitro. Furthermore, in a syngeneic tumor model of B16 melanoma we observed a >50% reduction in the volume of irradiated tumors in a CD47-/- host. Immunohistochemical staining shows increased intratumoral CD8+ cytotoxic T-cells and activation of the granzyme-B/perforin pathway that could mediate the increased target cell death. Deficiency in CD47 selectively protects T-cells from IR induced death through increased expression of the autophagy related genes beclin1, ATG5, ATG7, LC3 and degradation of p62. Our findings indicate that agents targeting CD47 may allow for more aggressive application of radiation in the treatment of cancer and may increase the percentage of curative responses.

Oral Presentation

Common genetic variants in the PSCA gene influence gene expression and bladder cancer risk

Yi-Ping Fu[1], Indu Kohaar[1], Nathaniel Rothman[2], Julie Earl[3], Jonine D. Figueroa[2], Yuanqing Ye[4], Nria Malats[5], Wei Tang[1], Luyang Liu[1], Montserrat Garcia-Closas[2,6], Brian Muchmore[1], Nilanjan Chatterjee[2], McAnthony Tarway[1], Manolis Kogevinas[7-10], Patricia Porter-Gill[1], Dalsu Baris[2], Adam Mumy[1], Demetrius Albanes[2], Mark P. Purdue[2], Amy Hutchinson[11], Alfredo Carrato[12], Adonina Tardn[8,13], Consol Serra[14], Reina Garcia-Closas[15], Josep Lloreta[16], Alison Johnson[17], Molly Schwenn[18], Margaret R. Karagas[19], Alan Schned[20], W. Ryan Diver[20], Susan M. Gapstur[20], Michael Thun[20], Jarmo Virtamo[21], Stephen J. Chanock[1], Joseph F. Fraumeni, Jr.[2], Debra T. Silverman[2], Xifeng Wu[4], Francisco X. Real[3,14], and Ludmila Prokunina-Olsson[1]

[1]Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, NCI, NIH, Bethesda, MD; [2]Division of Cancer Epidemiology and Genetics, NCI, NIH Bethesda, MD; [3]Epithelial Carcinogenesis Group, Molecular Pathology Programme, Centro Nacional de Investigaciones Oncolgicas (CNIO), Madrid, Spain; [4]Genetic and Molecular Epidemiology Group, Centro Nacional de Investigaciones Oncolgicas (CNIO), Madrid, Spain; [5]Department of Epidemiology, University of Texas MD Anderson Cancer Center, Houston, TX; [6]Division of Genetics and Epidemiology, The Institute of Cancer Research, London SW7 3RP, UK; [7]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain; [8]Municipal Institute of Medical Research, Barcelona, Spain; [9]CIBER Epidemiologa y Salud Pblica (CIBERESP), Barcelona, Spain; [10]National School of Public Health, Athens, Greece; [11]Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [12]Ramn y Cajal University Hospital, Madrid, Spain; [13]Instituto Universitario de Oncologa, Universidad de Oviedo, Oviedo, Spain; [14]Departament de Cincies Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain; [15]Unidad de Investigacin, Hospital Universitario de Canarias, La Laguna, Spain; [16]Hospital del Mar-Institut Municipal dInvestigaci Mdica (IMIM), Universitat Pompeu Fabra, Barcelona, Spain; [17]Vermont Cancer Registry, Burlington, VT; [18]Maine Cancer Registry, Augusta, ME; [19]Dartmouth Medical School, Hanover, NH; [20]Epidemiology Research Program, American Cancer Society, Atlanta, GA; [21]National Institute for Health and Welfare, Helsinki, Finland

Genome-wide association studies (GWAS) have identified a single nucleotide polymorphism (SNP), rs2294008, on 8q24.3 within the prostate stem cell antigen (PSCA) gene, as a risk factor for bladder cancer. To fine-map this region, we imputed 642 SNPs within 100Kb of rs2294008 in addition to 33 markers genotyped in one of the reported GWAS in 8,652 subjects. A multivariable logistic regression model adjusted for rs2294008 revealed a novel signal, rs2978974 (r2=0.02, D=0.19 with rs2294008). In the combined analysis of 5,393 cases and 7,324 controls, we detected a per-allele OR=1.11 (95%CI=1.06-1.17, p=5.8 10-5) for rs2294008 and OR=1.07 (95%CI=1.02-1.13, p=9.7 10-3) for rs2978974. The effect was stronger in carriers of both risk variants (OR=1.24, 95%CI=1.08-1.41, p=1.810-3) and there was a significant multiplicative interaction (p=0.035) between these two SNPs. The T risk allele of rs2294008 was associated with increased PSCA mRNA expression in two sets of bladder tumor samples (n=36, p=0.0007 and n=34, p=0.0054) and in normal bladder samples (n=35, p=0.0155), while rs2978974 was not associated with PSCA expression. SNP rs2978974 is located 10Kb upstream of rs2294008, within an alternative non-translated first exon of PSCA. The non-risk allele G of rs2978974 showed strong interaction with nuclear proteins from five cell lines tested, implying a regulatory function. In conclusion, a joint effect of two PSCA SNPs, rs2294008 and rs2978974, suggests that both variants may be important for bladder cancer susceptibility, possibly through different mechanisms that influence the control of mRNA expression and interaction with regulatory factors.

#58

Identification of protein kinase C activating phorbol ester and ingenol derivatives as candidates for cutaneous P-glycoprotein absorptive transport across the epidermis

Megan Ansbro[1], Luowei Li[1], Suneet Shukla[2], Suresh Ambudkar[2], Peter Blumberg[1], and Stuart Yuspa[1]

[1]Laboratory of Cancer Biology and Genetics, NCI, Bethesda, MD; [2]Laboratory of Cell Biology, NCI, Bethesda, MD

P-glycoprotein (P-gp/MDR1/ABCB1) is a membrane-associated multidrug transporter of the ABC transporter family that facilitates the multidrug resistance of cancer cells. P-gp is expressed in normal tissue including the skin, liver, kidneys, and serves a vital role in determining the distribution of drugs within the inter- and intracellular environment. Protein kinase C (PKC), a family of serine and threonine protein kinases, is often a target for anticancer therapies. We recently reported that Ingenol 3-angelate (Ing3A), a PKC agonist currently in clinical trials for topical treatment of squamous cell carcinoma, basal cell carcinoma, and actinic keratosis, acts via a P-gp dependent mechanism when applied topically in mouse models. In contrast with phorbol 12-myristate 13-acetate (PMA) topical treatment, in which PKC activation remains localized in the epidermis, Ing3A spans through the epidermis via P-gp mediated skin absorptive transport to activate PKC in the dermis. This potent PKC activation damages tumor vasculature and establishes the use of an active P-gp mechanism as a novel therapeutic treatment for cancer. In our current study, 11 phorbol ester and ingenol derivatives were evaluated for their ability to function as P-gp substrates/inhibitors. P-gp inhibitory activities of the compounds were measured by calcein AM efflux assays using P-gp overexpressing KB-V-1 cells or HeLa cells transduced with a P-gp expressing BacMam virus. The compounds were also tested for their ability to shift drug sensitivity to doxorubicin in HCT-15 P-gp overexpressing cells. Three phorbol ester derivatives and one ingenol derivative displayed P-gp inhibitory activities. Direct interaction of the four compounds with P-gp was demonstrated in a photoaffinity labeling assay in which the derivatives blocked the substrate [125I]iodoaryazidoprazosin (IAAP) from binding P-gp. In silico analysis of the derivatives calculated hydrophilicity of each compound and computed LogP values ranging from -0.56 to 8.78. P-gp substrate/inhibitor activities of the four compounds displayed no correlation in hydrophilicity. Through this study, we have identified a subfamily of PKC activators that also function as P-gp substrates/inhibitors. These compounds are new therapeutic candidates for P-gp mediated skin absorptive transport.

#59

PDGFR activation defines a subset of metastasis-associated astrocytes in the neuro-inflammatory microenvironment of breast cancer brain metastasis, inhibitable by pazopanib

Brunilde Gril[1], Diane Palmieri[1], Yong Qian[1], Talha Anwar[1], David J. Liewehr[2], Seth M. Steinberg[2], Patricia S. Steeg[1], and Fernando Vidal-Vanaclocha[3]

[1]Womens Cancers Section, Laboratory of Molecular Pharmacology, CCR, NCI, Bethesda, MD; [2]Biostatistics and Data Management Section, CCR, NCI, NIH, Rockville, MD; 3CEU-San Pablo University School of Medicine Urb. Monteprncipe, Boadilla del Monte, Madrid, Spain; [3]Biostatistics and Data Management Section, CCR, NCI, NIH, Bethesda, MD

Brain metastases of breast cancer occur in over a third of metastatic breast cancer patients whose tumors overexpress HER2 or are triple negative. The brain offers a unique environment, containing microglia, oligodendrocytes, astrocytes and neurons. While a neuroinflammatory response has been documented in brain metastasis, its contribution to cancer progression and therapy remains poorly understood. Using a model system to study experimental brain metastasis of breast cancer, we intensively characterized the brain metastatic microenvironment of brain tropic, HER2 transfected MDA-MB-231 human breast carcinoma cells (231-BR-HER2). A previously unidentified subpopulation of metastasis-associated astrocytes expressing phosphorylated PDGFRβ was identified surrounding brain metastases. Previously, we reported that pazopanib, a multispecific tyrosine kinase inhibitor targeting VEGFR-1, -2, and -3, PDGFR-α and β, and c-kit, directly targeted tumor cells through the disruption of B-Raf pathway, preventing the outgrowth of 231-BR-HER2 large brain metastases by 73% ($p<0.0001$). Here we evaluated the effect of pazopanib on the brain neuro-inflammatory microenvironment. Pazopanib treatment resulted in a 72% ($p=0.023$) decrease of the pY751 PDGFRβ + astrocyte population, at the lowest dose of 30mg/kg, twice daily. Collectively, we identified a new mechanism of action of pazopanib as an inhibitor of the newly identified population of metastasis- associated astrocytes. Pazopanib may represent a potent therapeutic approach, targeting both cancer cells and the microenvironment.

#60

A comprehensive analysis of demographic, clinical, laboratory and patient reported markers of oral chronic graft-versus-host disease

Helen Fassil, MPH[1,2], Carol Bassim, DMD, MS[3], Dean Edwards, DMD[3], Jacqueline Mays, DDS, PhD[3], Kristin Baird, MD[4], Seth M. Steinberg, PhD[5], Kirsten M. Williams, MD[1], Edward W. Cowen, M., MHSc[6], Sandra A. Mitchell, PhD[7], Fran T. Hakim, PhD[1], Tiffani Taylor, CRNP[1], Daniele Avila, CRNP[1], Dan Zhang, MS[5], Lana Grkovic, MD[1,8], Manuel Datiles, MD[9], and Steven Z. Pavletic, MD[1]

[1]Experimental Transplantation and Immunology Branch, NCI, NIH, Bethesda, MD; [2]Tufts University School of Dental Medicine; [3]National Institute of Dental and Craniofacial Research, Boston, MA; [4]Pediatric Oncology Branch, NCI; [5]Biostatistics and Data Management Section, Office of the Clinical Director, CCR, NCI; [6]Dermatology Branch NCI; [7]Division of Cancer Control and Population Sciences, NCI; [8]Division of Hematology, Department of Internal Medicine, Clinical Hospital Center Zagreb, Zagreb, Croatia, National Institute of Dental and Craniofacial Research; [9]National Eye Institute, NIH, Bethesda, MD

Chronic graft versus host disease (cGVHD) is a complication of allogeneic hematopoietic stem cell transplantation (alloHSCT). Oral cGVHD is manifested by mucosal, salivary and/or sclerotic changes that have been linked to pain and poor quality of life. Our aim was to describe the demographic, clinical, laboratory and patient reported markers of oral cGVHD in alloHSCT patients (N=187) enrolled in a cGVHD natural history study at the NIH (#NCT00331968). We used the NIH 15-point oral cGVHD activity assessment scale to define the cutoff point of clinical minimally detectable cGVHD (0-2=no oral cGVHD, 3-15=oral cGVHD). Forty-four (23.5%) patients had oral cGVHD. Oral cGVHD was associated with quiescent or de-novo type of cGVHD onset (p=0.05), higher NIH average cGVHD organ severity score (p=0.033), lower albumin (p=0.0008) higher total complement (p=0.012), greater bother from avoidance of foods or oral ulcers and greater mouth pain and sensitivity (p<0.0001). Multivariable logistic regression modeling using albumin, mouth pain and sensitivity was 74.3% predictive of oral cGVHD and 80.2% predictive of no oral cGVHD. We propose the use of >2 points on the NIH scale as a reproducible definition of clinically significant oral cGVHD which may be useful in clinical settings or as an endpoint in clinical trials.

#61

Clinical value of F-18 sodium fluoride PET/CT in advanced prostate cancer

Joseph W. Kim[1], Liza Lindenberg[2], William Dahut[1], James Gulley[1], Ravi Madan[1], Lauren V. Wood[1], Peter Choyke[2], Karen A. Kurdziel[2], Andrea B. Apolo[1]

[1]Medical Oncology Branch, CCR, NCI; [2]Molecular Imaging Program, CCR, NCI

Background: We evaluated the clinical utility of 18F-sodium fluoride PET/CT bone scan (18F-NaF) in the detection of bone metastases in patients (pts) with prostate cancer in comparison with Technetium-99m MDP bone scan (TcBS) and its impact on clinical management. Methods: In a prospective study, from October 2010-December 2011, 30 prostate cancer pts (ages 51-79), 21 with known bone metastases and 9 without known bone metastases, had 18F-NaF and a TcBS performed. Abnormal foci of uptake on both TcBS and 18F-NaF were classified as benign, malignant or indeterminate. Benign lesions included uptake in the joints and linear uptake at the endplates of the vertebral bodies consistent with degenerative changes. Malignant uptake on 18F-NaF scans was confirmed by characteristic osteoblastic features on CT. All TcBS and 18F-NaF were reviewed by an experienced nuclear medicine physician. For the patient-based analysis, scan results were categorized as positive (POS) = any malignant lesion; indeterminate (IND) = not distinctly malignant or benign; negative (NEG) = benign lesions only. Results: In the lesion-based analysis, 21 of 30 (70%) pts had more malignant lesions identified on 18F-NaF than on TcBS. The mean number of additional malignant lesions per patient on 18F-NaF vs TcBS was 4. Eight of 30 pts had same number of malignant lesions identified in both studies. One of 30 pts had one less malignant lesion identified on 18F-NaF than on TcBS. CT correlation by 18F-NaF PET/CT of this particular lesion did not confirm osteoblastic feature. Malignant lesion distribution on 18F-NaF included: spine (28% of lesions), thorax (26%), pelvis (24%), long bones (13%) and skull (10%). In the patient-based analysis, 24 pts (80%) were POS by 18F-NaF, of whom 14 pts were POS, 8 were IND, and 2 were NEG by corresponding TcBS; in the 4 pts with NEG 18F-NaF, zero were POS and 2 were IND and 2 were NEG by corresponding TcBS. Conclusion: 18F-NaF identified more malignant lesions than TcBS. 18F-NaF may also add useful information in the management of prostate cancer pts with and without known bone metastases.

#62

A Pharmacokinetic/Pharmacodynamic Study with Phase I Run-in with a PARP Inhibitor (Olaparib) in Combination with Carboplatin for Refractory or Recurrent Womens Cancers

David Moorshead*, Mario Cedillo, John Hays, Nicole Houston, Jung-min Lee, and Elise Kohn
Medical Oncology Branch, NCI, NIH

Ovarian cancer is the most lethal gynecologic cancer and is the fifth leading cause of cancer-related death among women. Recurrent platinum-sensitive ovarian cancer has been traditionally treated with a platinum-based chemotherapy doublet such as carboplatin with a taxane or cisplatin with gemcitabine. Novel targeted agents such as the poly (ADP-ribose) polymerase (PARP) inhibitor, olaparib (O), combined with carboplatin (C) have shown promising results in early-phase clinical trials. We hypothesized that there are differences in clinical activity with the sequential use of C with O. In preclinical testing we examined sequence specificity of C and O combinations in four cell lines, including two BRCA1mut (HCC1937, UWB1.289) and two BRCA1-WT lines (OVCAR8, HEYA8), on the development of DNA damage and cell injury. These experiments demonstrated differential cytotoxicity by XTT assay as well as differential DNA damage by comet assay and immunofluorescence. However, platinum incorporation as measured by atomic absorption spectroscopy was not altered in the presence of O exposure. Based on the preclinical data, we designed a clinical trial to measure pharmacokinetic and pharmacodynamic effects of the sequencing of C and O in patients with recurrent womens cancers. The first stage is a phase I dose escalation study to determine the safe Q12h dose of O with C, and the second stage is an expansion cohort consisting of two arms randomized to treatment of C followed by O or O followed by C. We validated the freezing and use of normal volunteer peripheral blood mononuclear cells (PBMCs) for comet assay. Afterward, we developed a rapid comet assay protocol to test levels of DNA damage on patients PBMCs. Tumor biopsies prior, during, and after treatment with individual agents or combinations have shown responses, but further translational analysis is needed to confirm what we have demonstrated in preclinical models.

#63

Complementary and Alternative Medicine Use by Hispanic Cancer Patients and the Impact of Acculturation: Results from the 2001 California Health Interview Survey and 2007 National Health Interview Survey

Jeans M. Santana[1], Justice Mbizob[2], Farah Zia[1], Oluwadamilola Olaku[1], Mian B. Hossainb[2], and Jeffrey D. White[1]

[1]Office of Cancer Complementary and Alternative Medicine, 6116 Executive Blvd. Suite 609, Rockville, MD;
[2]University of West Florida School of Allied Health and Health Sciences, 11000 University Parkway, Pensacola, FL

INTRODUCTION: Extensive research has found that CAM-use is prevalent among those diagnosed with cancer. For the Hispanic population, cancer is the second leading cause of death, accounting for 20% of all deaths. Among the entire US Hispanic population, the majority of the adult Hispanic population is foreign born. OBJECTIVE: To estimate the prevalence of CAM-use among US- and foreign-born Hispanic adults diagnosed with cancer and analyze how acculturation might affect CAM-use. METHODOLOGY: Data for the study was abstracted from the complete 2001 California Health Interview Survey (CHIS) and 2007 National Health Interview Survey (NHIS) using STATA version 11.0. RESULTS: There were 210 Hispanic cancer patients in the 2001 CHIS-CAM sample; and statistical analyses are still in progress. The 2007 NHIS-CAM dataset contained 129 Hispanic cancer patients. Fifty-one (51%) are foreign-born and those patients between ages 60 and 69 are more likely to use CAM. The most commonly used is physical activity (19%) followed by manipulative and body-based therapies (16%) and mind-body interventions (9%). Use of pharmacological therapies is low, accounting for 3%. Of the respondents 68% reside in the south and west regions. CONCLUSION: CAM-use is high among cancer patients. Across the different modalities, the use of dietary or pharmacological therapies is considerably less than that of non-pharmacological approaches. Specific Hispanic cancer patient data for CHIS is still in progress and at the moment conclusions regarding the prevalence of CAM use and the affect acculturation may have is unknown. For the 2007 NHIS dataset, we were unable to identify the key determinants of CAM-use among the different subgroups and the effect of acculturation. More funding is warranted to better understand: 1) Hispanic health-seeking behaviors; 2) safety and efficacy of CAM; and 3) issues surrounding CAM and conventional treatment interaction.

#64

Identifying HIV Transmission Networks in the Washington DC Metropolitan Area Using Phylogenetic Techniques

Tiffany Tanzosh1, Eleanor Wilson4, Robin Dewar2, Tauseef Rehman2, Catherine Rehm3, Irini Sereti4, Shyam Kottilil4, and Frank Maldarelli1

1HIV Drug Resistance Program, NCI, Frederick, MD; 2Collaborative Clinical Resource Branch, SAIC-Frederick, NCI, Frederick, MD; 3Clinical Research Section, NIAID, NIH, Bethesda, MD; 4Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD

The Washington DC metropolitan area has the highest HIV prevalence in the United States. Current estimates indicate 3.2% of adults and adolescents (>12 years) are infected with HIV. Substantial transmission persists in traditional risk groups, including men who have sex with men (MSM), intravenous drug users (IVDU), and unprotected heterosexual sex, but detailed understanding of transmission networks and HIV epidemiology in the Washington DC area is lacking. Additional information will help improve efforts to expand testing and prevent further transmission. The recent development of bioinformatic approaches to analyze HIV sequences has been useful in identifying HIV transmission clusters. Combined with demographic data, such information provides new and useful understanding of epidemic trends. In order to investigate transmission networks in the Washington DC metropolitan area, we analyzed demographic data and HIV genotypic information from 218 patients newly diagnosed with HIV infection, including 32 patients with documented recent (<1 year) infection. Genotypes were aligned using MEGA 5.05, and alignments were subjected to Bayesian Monte Carlo Markov Chain analyses using BEAST v1.6.2. to create phylogenetic reconstructions. Phylogenetic branches with strong posterior probabilities (≥0.95) were designated as transmission groups. In this analysis, 16 transmission groups were identified; all of size 2-3 individuals. Transmission groups were largely segregated by risk and race, although one cross-risk transmission was documented. Nearly a third of known recent infections were associated with a transmission group. Comparable studies in other metropolitan areas identified more transmission networks. Our data suggests a diffuse network of HIV transmission in the Washington, DC metropolitan area. Larger sample sizes will be necessary to detect more transmission networks.

Signal Transduction, Transcription, and Chromatin

Oral Presentation

Multiple Chromatin Remodeling Systems Contribute to Dynamic Transitions in Chromatin Structure

Stephanie A. Morris, Songjoon Baek, R. Louis Schiltz, Myong-Hee Sung, Malgorzata Wiench, Sam John, and Gordon L. Hager

Laboratory of Receptor Biology and Gene Expression, CCR, NCI

ATP-dependent chromatin remodeling is an essential process required for the dynamic organization of chromatin structure. However, the in vivo functions and interactions of remodeler proteins involved in this process are poorly understood. Here, we mapped the genome-wide location of three remodeler proteins with diverse physiological functions in the mouse genome: Brg1, Chd4, and Snf2h. Using chromatin immunoprecipitation combined with high throughput sequencing, we found these proteins localize to distinct sites within the genome in both inter- and intragenic regions. Binding patterns indicate remarkable overlap between each of the remodeler systems at many sites throughout the genome. These remodeler binding patterns closely mimicked the DNase I hypersensitivity profile, emphasizing the link between accessible chromatin and remodeler proteins. Finally, by expressing mutant variants, we were able to directly assign remodeling activity at individual sites, and demonstrate each remodeler contributes both to chromatin opening and closing. Surprisingly, we identified many regions of accessibility that require the concerted actions of all three remodelers. Thus, we propose a general mechanism where the organization of nucleosomes is a dynamic process requiring the activity of multiple remodeling systems. Collectively, these findings provide a complex view of chromatin organization and highlight the differential contributions of remodelers to chromatin maintenance in higher eukaryotes.

Oral Presentation

Analysis of DNA Replication in Cancer Cells upon Knockdown of Licensing and Initiation Factors

Elisabetta Leo, Kevin Yan, and Yves Pommier

Laboratory of Molecular Pharmacology, CCR, NCI

Chromosome replication occurs precisely once during the cell cycle, and is a highly complex process that is still relatively poorly understood. The regulation of licensing, activation and firing of the origins scattered along the genome is crucial for maintaining genomic stability: incomplete replication, as well as re-replication leads to double strand breaks, a hallmark for cancer. The aim of this work was to understand how cells regulate early stages of DNA replication and respond to its alterations. We treated two human cancer cell lines and one non transformed cell line with siRNAs targeting a pre-replication component Cdc6, the origin activator Cdc7, and the pre-initiation complex component Cdc45, also known as limiting factor for replication initiation. We monitored: the alteration of cell cycle profile and BrdU incorporation, the G1/S transition and the activation of DNA damage and performed detailed studies on DNA replication pattern. We found striking differences in the way the replication machinery acts after depleting the three factors. In particular, normal cells were able to detect the misregulation of licensing/initiation and arrest in G1. On the contrary, cancer cells initiated S-phase and seemed unable to detect the alterations until much later, and consequentially die. Our studies also revealed a distinct response of the cancer cells to the block of origin licensing, activation or firing steps. In all the cases, replication pattern was grossly altered and a mutual regulation of fork progression and inter origin distances was observed. In particular, when Cdc45 was silenced, we observed replication fork slowdown and overall reduction in the DNA synthesis but, also activation of dormant origins within replication clusters consisting on average of 3-6 origins 35 kb far apart. These observations shed light on the mechanisms that regulate the early stages of DNA replication and the different ways cells respond to its alterations.

Oral Presentation

C/EBPδ promotes nuclear localization of p21CIP1 and cytotoxicity of tamoxifen in ER(+) breast tumor cells

Su-Ryun Kim[1], H. Raza Ali[2], Shikha Sharan[1], Carlos Caldas[2], and Esta Sterneck[1]

[1]CCR, NCI, Frederick, MD; [2]University of Cambridge, Cambridge, UK

The transcription factor CCAAT/enhancer binding protein delta (C/EBPδ) is expressed in normal breast epithelial cells and down-regulated in breast cancer tissue and cell lines (Porter et al., 2003; Sarkar et al., 2012). In the MMTV-Neu mouse mammary tumor model, loss of C/EBPδ leads to increased tumor incidence while reducing tumor metastasis (Balamurugan et al., 2010). To investigate the role of C/EBPδ in human breast cancer, we analyzed tissue microarrays by immunostaining. We found that the C/EBPδ protein is expressed in 50% of invasive estrogen receptor α positive ER(+) breast carcinomas, which is associated with longer survival of ER(+) cancer patients, independent of tumor grade. Anti-estrogenic treatment is a first-line therapy for patients with ER(+) cancer. Therefore, we investigated the role of C/EBPδ in the response of breast tumor cells to the ER inhibitor tamoxifen (TAM). RNAi-mediated silencing of C/EBPδ increased the survival of TAM treated MCF-7 cells, suggesting that C/EBPδ contributes to the cytotoxicity of TAM. Nuclear localization of the CDK inhibitor p21CIP1 is necessary for inhibition of cell growth/survival by TAM, and cytoplasmic p21CIP1 in tumors is associated with poor prognosis (Abukhdeir et al., 2008; Xia et al., 2004). We found that C/EBPδ depletion led to cytoplasmic localization of p21CIP1. Furthermore, C/EBPδ silencing increased the phosphoylation level of ERK kinases, which had was previously been shown to promote cytoplasmic localization of p21CIP1 (Heo et. el., 2011). Preliminary data indicate that a constitutively nuclear mutant form of p21CIP1 rescues the TAM-response of C/EBPδ-silenced MCF-7 cells. These results suggest that a target gene of C/EBPδ is necessary to inhibit ERK phophorylation and thereby promotes nuclear localization of p21CIP1 and TAM toxicity. Future analyses will address the mechanism for ERK inhibition by C/EBPδ, and investigate the correlation of C/EBPδ expression with p21CIP1 localization in tumor tissue.

Oral Presentation

Bidirectional Transcription of the Upstream Regulatory Region may serve a Regulatory Role in MHC Class I Transcription

Aparna Kotekar and Dinah Singer

Molecular Regulation Section, Experimental Immunology Branch, NCI, Bethesda, MD

The major histocompatibility complex I (MHC Class I) gene encodes a protein involved in presenting antigens to T cells, a process crucial to immune surveillance with implications in tumor immunity, immunity to intracellular pathogens, graft versus host disease and autoimmunity. Our laboratory is focused on dissecting the mechanisms of regulation of this ubiquitously expressed gene that displays both tissue-specific and hormonal-/cytokine-mediated transcriptional changes. In the course of studying the chromatin structure of the class I gene and investigating the implications of qualitative chromatin differences to transcriptional control, we discovered histone modifications in upstream regulatory regions that suggested that this region was transcribed. Using real time RT-PCR, we have shown that there is indeed transcription occurring from the upstream regulatory regions of the MHC class I gene. These upstream regions are traditionally thought of as non-transcribed regions. Using strand-specific reverse transcription, we have demonstrated that these upstream transcripts arise both from the sense and anti-sense strands. Furthermore, our investigations have revealed bidirectional transcription from the class I core promoter. Interestingly, upstream transcript levels were regulated in a tissue-specific fashion and correlated positively with transcription of the class I message. Furthermore, induction of the class I gene with Interferon- gamma resulted in complex changes in upstream transcript profiles suggesting that these upstream transcripts could be involved in dynamic regulation of class I transcription. Such unconventional non-coding transcripts resulting from bidirectional transcription from promoters or arising from upstream regulatory regions of genes have only recently been recognized in eukaryotic systems as a potential transcriptional regulatory mechanism. How these transcripts might regulate transcription of the transcription units they arise from is largely unknown. We have designed and constructed a unique plasmid vector that would help to characterize bidirectional promoters. Currently, we are investigating the role(s) of upstream non-coding RNA in class I

transcription regulation.

Oral Presentation

A mammalian replicator binding protein essential for sequence-specific initiation of DNA replication

Liang Huang[1], Ya Zhang[1], Haiqing Fu[1], Chii Mei Lin[1], Qiaoming Long[2], and Mirit I. Aladjem[1]

[1]Laboratory of Molecular Pharmacology, CCR, NCI, NIH Bethesda, MD; [2]Department of Animal Science, Cornell University, Ithaca, NY

Mammalian DNA replication initiates at discrete sites irregularly spaced along chromosomal DNA molecules, but the principles governing the choice of replication initiation sites are unclear because proteins that are essential for DNA replication do not bind to specific DNA sequences. We have identified a replication initiation determinant (Rep-ID) protein that binds to a subset of mammalian replication initiation sites. Rep-ID is a member of the Ddb1- and Cul4-associated factor family (DCAF14/PHIP), and mainly localizes in the nucleus. The levels of Rep-ID peak at the G1 phase of the cell cycle and decrease during S phase. ChIP-seq data show that Rep-ID binds a set of mammalian replicators and facilitates replication initiation. Rep-ID depleted cells show fewer replication initiation events and abnormal replication fork progression. At early replicating human beta globin loci, Rep-ID binding is required for DNA replication from the RepP replicator. In K562 cells, Rep-ID interacts with Cdt1, a pre-replication complex member, before replication initiates. ChIP-3C results indicate that Rep-ID participates in an essential distal interaction between the human beta globin locus replicator, RepP, and the locus control region that regulates replication and transcription. These observations are consistent with a model that Rep-ID facilitates replication initiation in a distinct subset of replication origins.

#65

Core Promoter Elements Are Not Essential For Transcription In Vivo

Zohar S. Barbash, Jocelyn D. Weissman, Jie Mu, Dinah S. Singer

Experimental Immunology Branch, CCR, NCI

Core promoter elements were shown to be crucial in transcription regulation. We aim to identify the MHC class I core promoter elements that determine promoter activity and/or tissue specificity, by analyzing the expression of genomic transgenes of MHC-I genes, mutated in various core promoter elements. WE USED series of transgenic lines containing full length swine PD1 gene but differed in the presence of single promoter element mutation, in sequences homologous to INR, SP1 TATA and CAAT elements. WE FOUND surprisingly that all of the transgenes with mutated core promoter elements were capable of expression on mice PBL surface. We used RTQ PCR and Northern Blot to show that in TATA mutated mice there is overall higher transgene expression in all tissues, whereas INR and CAAT homologous elements displayed different patterns of expression compared to the WT. in contrast SP1 expression pattern was similar to WT expression pattern. We also found that in the INR and SP1 mutants the response to extracellular signaling is aberrant. The CAAT mutated strains exhibit a unique heritage pattern in subsequent generations. These strains were shown to have altered chromatin structure, suggesting a role of CAAT element as n insulator. Remarkably, these results demonstrate that none of the elements homologous to canonical core promoter elements are necessary for promoter activity. However, they do contribute to the fine tuning of the tissue specific patterns of expression, extracellular signaling and overall promoter activity.

#66

Phenotypic Outputs of Ovarian Cancer Cell Lines and Their Relationship to a Subset of the Signaling Kinome

Saawan Mehta[1], Joyce Lu[2], Elise C. Kohn[1], and John L. Hays[1]

[1]Medical Oncology Branch, NCI, NIH; [2]Albany Medical College, Albany, NY

Epithelial ovarian cancer is the most common cause of death among women with gynecological malignancies. Targeted therapy has provided some benefit in early stage clinical trials; however, it is unclear what are the proper targets in ovarian cancer. Partial least squares regression (PLSR) is a statistical method for generating predictive models when analyzing large datasets, such as those from genomic or proteomic analysis. We used four ovarian cancer cell lines: OVCAR8, CAOV3, SKOV3, and HEYA8 in assays designed to assess multiple aspects of ovarian cancer: cytotoxicity assay, the ability of tumor cells in co-culture to mitigate differentiation of microvascular endothelial cells (HMVECs), and anoikis. We utilized eight kinase inhibitors against clinical targets: XL147 (PI3K), vandetanib (EGFR), dasatinib (SRC), AZD6244 (MEK), rapamycin (mTOR), PF573228 (FAK), sunitinib (VEGFR), and AZD4054 (ETAR). We then will measure the proteomic changes in the tumor cells using reverse phase protein array (RPPA) of a selected fraction of the kinome to generate the datasets for PLSR analysis. We hypothesize that PLSR of the proteomic datasets with the measured phenotypic endpoints will provide a testable predictive model for targetable nodes in ovarian cancer. In cytotoxicity measurements, dasatinib was the most potent inhibitor (IC50 range 0.027-2.4 μM), while PF573228 showed little activity. All cell lines induced HMVEC differentiation in the co-culture assay. There, PF573228 inhibited differentiation at <1nM in all cell lines, while other drugs showed varied responses. We measured the sensitivity of HEYA8 cells to anoikis using a time course with all eight drugs and observed variable responses; the other three cell lines are being tested currently. A selected fraction of the kinome is currently being analyzed by RPPA. The data from the proteomics analysis will be used to help correlate the phenotypic response to the proteomic changes in the kinome.

#67

LAT-Independent Erk Activation via Bam32-PLC-γ1-Pak1 Complexes: GTPase-Independent Pak1 Activation

Alexandre K. Rouquette-Jazdanian[1], Connie L. Sommers[1], Deborah K. Morrison[2], and Lawrence E. Samelson[1]

[1]Laboratory of Cellular and Molecular Biology, CCR, NCI, NIH, Bethesda, MD; [2]Laboratory of Cell and Developmental Signaling, CCR, NCI, NIH, Frederick, MD

In T lymphocytes the adaptor Bam32 is coupled to Erk activation downstream of the TCR by an unknown mechanism. We characterized a novel pathway utilizing a Bam32-PLC-γ1-Pak1 complex, in which Pak1 kinase activates Raf-1 and Mek-1 both upstream of Erk. PLC-γ1(C-SH2) binds S141 of Bam32, preventing LAT-mediated activation of Ras by PLC-γ1. In the Bam32-PLC-γ1-Pak1 complex, catalytically inactive PLC-γ1 is used as a scaffold linking Bam32 to Pak1. The Bam32-PLC-γ1 interaction enhances the binding of the SH3 domain of the phospholipase with Pak1. The PLC-γ1(SH3)-Pak1 interaction activates Pak1 independently of the small GTPases Rac1/Cdc42, previously described as being the only activators of Pak1 in T lymphocytes. Direct binding of the SH3 domain of PLC-γ1 to Pak1 dissociates inactive Pak1 homodimers, a mechanism required for Pak1 activation. Additionally PLC-γ1(SH3) induces in vitro Pak1 super activation. We have thus uncovered a novel LAT/Ras-independent, Bam32-nucleated pathway that activates Erk signaling in T cells.

#68

Discovery and characterization of novel kinase activity in coactivator of transcription CIITA

Katherine Soe, Devaiah Ballachanda, and Dinah Singer

Experimental Immunology Branch, NCI, NIH, Bethesda, MD

Major Histocompatibility Complex (MHC) Class II Transactivator (CIITA) is a coactivator and general transcription factor that regulates both MHC Class I and Class II gene transcription, and is thus critical to activated immune response. Known as the master regulator of Class II expression, CIITA nucleates an enhanceosome complex to induce Class II transcription. A decrease in MHC Class II levels is linked to bare lymphocyte syndrome. In MHC Class I gene expression, CIITA nucleates a preinitiation complex to activate transcription. Comparison of eukaryotic basal transcription and interferon-activated transcription initiation reveals similar preinitiation complex recruitment mechanisms, and striking parallels between their respective critical components, the TATA-binding protein (TBP)-associated factor 1 (TAF1) and CIITA. Both TAF1 and CIITA have been shown to possess instrinsic acetyltransferase (AT) activity required to activate MHC transcription, which is regulated by TAF7. Moreover, CIITA can bypass the requirement for TAF1 to activate both the MHC Class I and II promoters. However, although TAF1 has kinase activity, no similar activity has been reported for CIITA thus far. In this study, we identify the transcriptional coactivator CIITA as a novel atypical serine-threonine kinase whose substrates include various general transcription factors. Additionally, we have characterized the kinase activity of the protein and mapped the putative kinase domains. We propose a model in which the kinase activity of CIITA serves a function similar to that of TAF1, in which it regulates TAF7 binding and release, and thus MHC transcription initiation. This may elucidate a novel role for CIITA in the regulation of activated transcription initiation and stimulated immune response during pathogenic infection.

#69

Characterization of Transcriptional Regulation of iPS-derived-Mesenchymal Stem Cells

Bethtrice Thompson[1,2], Lyuba Varticovski[1], Lars Grontved[1], and Gordon L Hager[1]

[1]Laboratory of Receptor Biology and Gene Expression, NCI-Bethesda; [2]Howard University

Induced pluripotent stem cells (iPS) have the ability to differentiate into any cell in the body, which is similar to the pluripotency observed in embryonic stem cells. iPS cells are generated from somatic cells by direct reprogramming with three or four specific transcriptional factors (Nanog, Oct4, Sox2, c-Myc, Klf4, and/or Lin28). It is essential to fully understand the global chromatin landscape that determines transcriptional regulation of differentiation. Moreover, the interaction between transcription factors and DNA during differentiation of iPS cells will lead to a better understanding of how specific protein complexes localize at specific genomic regions, and how these interactions regulate gene expression. Genome-wide identification of transcription factor binding has been previously assessed by Chromatin Immunoprecipitation (ChIP) in combination with deep sequencing (ChIP-seq). However, ChIP and ChIP-seq provide only a focused view of a specific target binding. Our laboratory pioneered analysis of DNase I hypersensitive sites (DHS) combined with deep sequencing (DHS-seq) to interrogate the landscape of the entire accessible genome (i.e. all sites in the genome that are accessible to transcription factors at any time). Objective: We propose to use this approach to characterize iPS cells and to identify the regulatory events of the human genome during their differentiation. Methods: iPS cells were grown on irradiated mouse embryonic fibroblast feeder layer (iMEF) or on Matrigel, and differentiated into embryonic bodies or mesenchymal stem cells and terminal differentiated into osteoblast. Results: Pluripotent cells were characterized by DHS-seq, gene expression by qPCR, immunohistochemistry, and flow cytometry. Future Work: Genome-wide chromatin accessibility will be compared to ENCODE data for human ES cells. Conclusion: Our study is the first genome-wide analysis of human iPS cells during differentiation, and our data will serve as a baseline for characterization of normal and aberrant bone formation.

#70

Glucocorticoid receptor interaction with the genome in mouse liver tissue is regulated by C/EBP and baseline chromatin accessibility

Lars Grontved[1], Sam John[1], Songjoon Baek[1], Ying Liu[3], John R. Buckley[2], Charles Vinson[2], Greti Aguilera[3], and Gordon Hager[1]

[1]Laboratory of Receptor Biology and Gene Expression CCR, NCI, NIH, Bethesda, MD; [2]Laboratory of Metabolism, CCR, NCI, NIH, Bethesda, MD; [3]Section on Endocrine Physiology, Program on Developmental Endocrinology and Genetics, National Institute of Child Health and Diseases, NIH, Bethesda, MD

Glucocorticoids are used for management of auto-inflammatory diseases and glucocorticoid receptor (GR) dysregulation is implicated in the pathogenesis of stress-related disorders and linked to chronic inflammation associated with the metabolic syndrome. How the glucocorticoid receptor targets specific regions of the genome to elicit physiological responses is a largely unanswered question. Here we disrupted GR activity in C57BL/6 mice by surgical removal of the adrenal glands, the organ responsible for GR ligand (glucocorticoids) synthesis. Three day post surgery, adrenalectomized mice were injected with the synthetic GR agonist, dexamethasone or vehicle one hour prior tissue harvesting. ChIP-seq analysis uncovered ~10,000 highly reproducible dexamethasone induced GR binding sites in liver and combined with DNase-seq we observe that 80% of GR binding sites fall into the baseline pre accessible chromatin compartment. De novo DNA motif analysis suggests a functional role of C/EBP supported by ChIP-seq against C/EBPbeta, where the majority of GR binding in the pre-accessible chromatin compartment is occupied by C/EBP. In addition a number of C/EBPbeta binding events are facilitated by GR binding to the genome in a de novo chromatin remodeling dependent and independent manner. In order to uncover the functional role of C/EBP we tail vain injected mice with adenovirus expressing dominant negative C/EBP (DN-C/EBP). Four days post injection we observe reduced C/EBP binding to chromatin and disrupted chromatin accessibility at C/EBP binding sites compared to control mice injected with adenovirus expressing GFP. At pre-accessible GR binding sites pre-occupied by C/EBP, DN-C/EBP disrupts maximum GR binding compared to control mice. Interestingly disruption of C/EBP occupancy also reduces GR recruitment to and chromatin remodeling of sites where C/EBP binding is assisted by GR, demonstrating a cooperative mechanism by which GR and C/EBP specifically access chromatin in liver.

#71

Modulation of the Mutant p53 Pathway in Metastatic Triple Negative Breast Cancer

Bethanie L. Morrison[1] and Federico Bernal[1]

[1]Laboratory of Cancer Chemical Biology, Metabolism Branch, CCR, NCI

The mutation of p53 generally occurs during the later stages of cancer and leads to the increased metastatic potential of triple negative (ER—/PR—/Her2—) breast cancer. The low survival rates of advanced metastatic cancer make it critical not only to understand the mechanisms underlying the functional implications of p53 mutation, but also to identify ways to regulate its impact in cancer cell physiology. We have previously described the design, synthesis, and evaluation of a stabilized α-helix of p53 (SAH-p53) capable of inhibiting the p53-HDM2 and p53-HDMX interactions in order to restore the functionality of p53. Despite having low expression of both HDM2 and HDMX and mutations in p53, SAH-p53 treatment of the triple negative breast cancer cell lines MDA-MB-231 and HS578T results in a modest decrease in proliferative activity. While mutant p53 is known to promote metastatic behavior of breast cancer cells, the propensity of triple negative breast cancer cells to migrate and invade through Matrigel in the presence of EGF was decreased by treatment with SAH-p53. The anti-proliferative and anti-migratory effects of SAH-p53 treatment are independent of each other, yet both are accompanied by an alteration in cell morphology that includes the loss of cell membrane integrity and actin cytoskeletal organization. The surface level of β1 integrin, which is typically kept constant by mutant p53, is decreased in the presence of SAH-p53, pointing to possible alterations in integrin recycling. Targeted therapy for triple negative breast cancer is currently very limited and patients must rely on standard chemotherapeutic regimens. These data show that the use of the SAH-p53 peptide provides a novel avenue to study the molecular interactions that drive metastatic behavior originated by p53 gain-of-function mutations.

Cancer Prevention, Carcinogenesis, and Cancer Stem Cells

Oral Presentation

The reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by c-MYC

Wei Liu[1], Anne Le[2], Chad Hancock[1], Andrew N. Lane[3], Chi V. Dang[4], Teresa W-M. Fan[3], and James M. Phang[1]

[1]Metabolism and Cancer Susceptibility Section, Basic Research Laboratory, CCR, NCI; [2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore; [3]Center for Regulatory Environmental Analytical Metabolomics (CREAM), University of Louisville, Louisville; [4]Abramson Cancer Center, University of Pennsylvania, Philadelphia

Growing tumors alter their metabolic profiles to meet the bioenergetic and biosynthetic demands of increased cell growth and proliferation. The oncogenic transcription factor c-MYC (MYC) has been linked to altered cellular metabolism of tumors. In addition to glycolysis, MYC stimulates glutamine catabolism to fuel growth and proliferation of tumor cells through upregulating glutaminase (GLS). Glutamine is converted to glutamate by GLS, which can enter the TCA cycle as an important energy source. Less well-recognized, glutamate can also be converted to proline through Δ1-pyrroline-5-carboxylate (P5C), and vise versa. Our current studies suggest that some of the cellular effects of MYC are due, in part, to the reprogramming of proline and glutamine metabolism. POX, the first enzyme in proline catabolism, is a mitochondrial tumor suppressor that inhibits proliferation and induces apoptosis. Our studies have shown that the downregulation of POX in human kidney tumors is due to increased miR-23b*. Using MYC-inducible P493 human lymphoma cells and PC3 human prostate cancer cells, we showed that MYC suppressed POX expression primarily through upregulating miR-23b*. The growth inhibition in the absence of MYC was partially reversed by knockdown of POX, indicating the importance of suppression of proline catabolism in MYC-mediated cellular effects. In contrast to its effect on POX, MYC markedly increased the enzymes of proline biosynthesis from glutamine, including P5C synthase and P5C reductase. Using 13C, 15N-glutamine as a tracer, proline from glutamine catabolism was shown to be markedly increased by MYC. Blockade of proline biosynthesis by knocking down P5C synthase or P5C reductase decreased glycolysis and cell growth induced by MYC. The metabolic link between glutamine and proline afforded by MYC emphasizes the complexity of tumor metabolism. Further studies of the relationship between glutamine and proline metabolism should provide a deeper understanding of tumor metabolism while enabling the development of novel therapeutic strategies.

Oral Presentation

Oncogenic TLR-MyD88 Signaling In Activated B-Cell-Like Diffuse Large B-Cell Lymphoma: Mechanism and Potential Therapeutic Strategies

Kian-Huat Lim[1], Vu Ngo[2], Ryan Young[2], Roland Schmitz[2], Sameer Jhavar[2], Wenming Xiao[2], and Louis Staudt[2]

[1]Medical Oncology Branch, CCR, NCI; [2]Metabolism Branch, CCR, NCI

The activated B-cell-like (ABC) subtype of diffuse large B cell lymphoma (DLBCL) is a molecular subtype of DLBCL which responds poorly to standard chemotherapy and is associated with poor prognosis. The clinical aggressiveness of the ABC DLBCL is largely attributed to the pathogenic activation of the pro-survival NF-κB signaling cascade. Using an "Archilles heel" RNAi screen, we previously found that MyD88, amongst many other genes, are uniquely required for survival of ABC DLBCL cell lines. We further found that MyD88, which encodes an adaptor protein essential for Toll-like receptor (TLR) signaling, is mutated in about 30% of ABC DLBCL tumor samples. Here, we showed that many of the missense MyD88 mutations, in particular the most frequent L265P mutation, are oncogenic and able to constitutively activate multiple signaling cascades such as the NF-κB, type-1 interferon, p38 and JAK/STAT pathways. Mechanistically, we found that the MyD88 L265P oncoprotein binds constitutively to TLR7 and TLR9, and greatly enhances the signaling output from these receptors. All ABC DLBCL cell lines that carry the MyD88 L265P mutation invariably requires TLR7 and/or TLR9 for survival. Concordantly, shRNA knockdown of known proteins essential for TLR7 and TLR9 trafficking and maturation such as UNC93B1, PRAT4A or CD14, or pharmacologic inhibition of these processes using z-FA-fmk or hydroxychloroquine effectively diminish MyD88 signaling and survival of ABC DLBCL cell lines. Furthermore, suppression of essential downstream kinase of the TLR-MyD88 cascade, IRAK4, by shRNA or kinase-inhibitors demonstrated selective toxicity towards the ABC DLBCL cell lines, suggesting IRAK4 as an ideal candidate for targeted therapy. Collectively, our data showed that hyperactivation the TLR-MyD88 pathway by MyD88 mutations is a novel driving oncogenic mechanism underlying ABC DLBCL, and provided molecular targets that could be explored as effective anticancer strategies.

Oral Presentation

A single-domain human antibody elicits potent antitumor activity by targeting an epitope in mesothelin close to the cancer cell surface

Zhewei Tang[1,2], Mingqian Feng[2], Wei Gao[2], Yen Phung[2], Min Qian[1], Dimiter S. Dimitrov[3], and Mitchell Ho[2]

[1]Institute of Biomedical Sciences, School of Life Sciences, East China Normal University, Shanghai, China; [2]Laboratory of Molecular Biology, CCR, NCI, NIH, Bethesda, MD; 3Nanobiology Program, CCR, NCI-Frederick, Frederick, MD

Mesothelin is an attractive cancer therapeutic target because it is highly expressed on multiple forms of human solid tumors. However, mesothelin-targeted antibody therapy is not well established. Complement dependent cytotoxicity (CDC), a major anti-cancer mechanism, has not been found in any therapeutic anti-mesothelin antibodies currently in preclinical or clinical development, due most likely to every known epitope being located far from the cell membrane. Here, we identified SD1, a novel engineered human antibody domain to mesothelin, by phage display technology. The SD1 domain recognizes an epitope of mesothelin close to the cell surface. To investigate SD1 as a potential therapeutic, we generated a recombinant human Fc (hFc) fusion protein and an immunotoxin by fusing SD1 to a bacterial toxin. Interestingly, the SD1-hFc protein exhibits strong CDC activity, in addition to antibody-dependent cell-mediated cytotoxicity, against mesothelin-expressing tumor cells. The SD1 immuntoxin inhibits both 2D and 3D growth of cancer cells ex vivo. Furthermore, the SD1-hFc protein causes significant tumor growth inhibition of tumor xenografts in mice. These results demonstrated that the SD1 human antibody has potential as a cancer therapeutic candidate, and may lead to novel antibody therapy targeting mesothelin-expressing tumors.

Oral Presentation

Epigenetic Regulation of CpG Promoters by the NF-κB Signaling Pathway in Pancreatic Cancer Stem Cells

Lei Sun[1], Lesley A. Mathews[1, 2], Stephanie M. Cabarcas[1,3], Xiaohu Zhang[4], Acong Yang[5], Ying Zhang[6], and William L. Farrar[1]

[1]Cancer Stem Cell Section, Laboratory of Cancer Prevention, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD; [2]National Center for Translational Therapeutics, National Chemical Genomics Center, Human Genome Research Institute, Rockville, MD; [3]Biology Department, Gannon University, Erie, PA; [4]Cancer Stem Cell Section, Laboratory of Cancer Prevention, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD; [5]Laboratory of Protein Dynamics and Signaling, CCR, NCI-Frederick, Frederick, MD; [6]Guang An Men Hospital of China Academy of Chinese Medical Sciences, Beijing, China

Pancreatic cancer is one of the most common causes of cancer death internationally. The diagnosis of early-stage pancreatic cancer often has a poor prognosis and the survival rate is quite low once it becomes advanced or metastatic. Epigenetic modifications such as DNA methylation play a significant role during both normal human development and cancer progression. We sought to investigate which genes are epigenetically regulated in the invasive population of pancreatic cancer cells that are also termed as cancer stem cells (CSCs). We conducted epigenetic arrays in both PANC1 and HPAC pancreatic cancer cell lines and compared the global DNA methylation status of CpG promoters in invasive cells (CSC population) to their non-invasive counterparts (non-CSC population). The differentially methylated genes were applied into Ingenuity pathway analysis and our results showed that the NF-κB pathway is one of the top activated pathways in invasive cells. In line with this, we determined that upon treatment with NF-κB pathway inhibitors, the invasive and migratory ability of total cells are significantly disrupted. Moreover, the SRY-box transcription factor SOX9, which is demethylated in invasive cells, is shown to play a crucial role in invasion of both cell lines. In addition, we found a potential NF-?B binding site located in the SOX9 promoter in Genomatix database. Interestingly, the NF-κB subunit p65 positively regulates SOX9 expression by binding to its promoter directly, which can be efficiently blocked by NF-κB inhibitors. Thus, our work establishes a link between the classical NF-κB signaling transduction pathway and the invasive properties of pancreatic CSCs. We believe our data can result in the identification of novel signals and molecules at an epigenetic level that can potentially be targeted in pharmaceutical investigations and clinical trials.

Oral Presentation

Epigenetic modulation of glioma stem-like cells promotes decreased expression of CD133 and induces differentiation

Cody D. Schlaff, BS, Anita Tandle, PhD, W. Tristram Arscott, BS, Ira Gordon, DVM, Philip Tofilon, PhD, and Kevin A. Camphausen, MD

Radiation Oncology Branch, NCI, NIH, Bethesda MD

Glioblastoma multiforme (GBM) is the most aggressive and common primary brain malignancy in adults, and is characterized by its highly invasive, angiogenic, and heterogenic qualities. Despite aggressive therapies with surgery, ionizing radiation and chemotherapy, the disease ultimately recurs. The existence of a subpopulation of cells that exhibit stem cell-like properties is hypothesized to recapitulate the tumor bulk, leading to tumor recurrence and progression. These stem-like cells, specifically CD133 positive cells, have been shown in vitro to exhibit radioresistance. Therefore, new therapies that can alter the CD133 expression profiles of CSCs may prove beneficial for treatment with concurrent and/or adjuvant radiotherapy. One such class of drugs are histone deacetylase inhibitors (HDACi). The over activity of HDACs combined with the inactivity of histone acetyltransferases leads to transcriptional repression, and is associated with tumorigenesis. Glioma stem-like cells treated as neurospheres with 3.0 mM sodium butyrate (NaBu), 10 μM vorinostat (SAHA) and 10 mM valproic acid (VA) were analyzed by flow cytometry and found to decrease expression of CD133 by 23.2% (SEM 0.9025 $p < 0.001$), 43.4% (SEM 1.764; $p < 0.001$) and 65.3% (SEM 2.596; $p < 0.001$), respectively, after 48 hours of continuous treatment. However, CD133 positivity returned within 24 hours after removing the treatment, indicating only transient epigenetic modulation of CD133 expression. Cell morphology suggested HDACi treatment induces cellular differentiation. Clonogenic analysis supported this hypothesis with a surviving fraction of 32.6% (SEM 3.3% ; $p < 0.005$) for 3.0 mM NaBu and 31.7% (SEM 7.5%; $p < 0.005$) for 10 μM SAHA. Overall, our current studies suggest that HDACi treatment decreases the expression of CD133 in glioma CSCs in vitro and may induce differentiation. Further confirmatory studies will be performed to determine the fate of these stem-like cells and the downstream effects HDACi treatments have on increasing radiosensitivty.

#72

Tumor suppressor PDCD4 inhibits NFκB dependent transcription in human glioblastoma cells

Soon-Kyung Hwang, Noriko Yoshikawa, Alyson R. Baker, Glenn Hegamyer,Matthew R. Young and Nancy H. Colburn

Laboratory of Cancer Prevention, CCR, NCI, Frederick, MD

PDCD4 was first described as a protein induced by apoptotic stimuli that acts as a tumor suppressor. PDCD4 plays an important role in regulating transcription and translation. Our previous research showed that overexpression of PDCD4 leads to decreased proliferation in glioblastoma-derived cell lines as well as decreased tumor growth in a glioblastoma xenograft model. Moreover, PDCD4 has been shown to positively influence tumor necrosis factor-induced activation of the transcription factor NF-κB in inflammatory cells. However, the effect of PDCD4 on NF-κB transcriptional activity is still unknown in cancer cells. NF-κB acts as an oncogenic driver in many cancer sites. We studied the effect of PDCD4 on NFκB-dependent transcriptional activity in malignant human glioblastoma cell lines. We stably overexpressed PDCD4 in U251 and LN229 cells. Our results showed that stable Pdcd4 expression inhibits NF-κB transcriptional activation measured by a luciferase reporter in U251 and LN229 cells. We investigated the molecular mechanisms by which PDCD4 inhibits NF-κB transcriptional activation. Our results showed that PDCD4 overexpression does not inhibit pathways upstream of NF-κ B including the activation of IKKalpha and IKKbeta kinases or the expression of NF-κB proteins p65 and p50. In addition, PDCD4 does not inhibit phosphorylation or degradation of IκBα, events needed for nuclear transport of p65 and p50. In contrast overexpression of PDCD4 inhibits translocation of p65/50 to the nucleus. We are currently elucidating the molecular interactions that explain the PDCD4-inhibited nuclear transport. These results suggest that PDCD4 can significantly inhibit NF-κB activity in glioblastoma cells and further elucidate the molecular mechanisms by which the PDCD4-mediated repression of NF-κB activity leads to suppression of glioblastoma tumorigenesis. Our results may provide novel opportunities for NF-κB targeted interventions to prevent or treat cancer.

#73

Myc-driven lymphomagenesis prevented by pharmacological inhibition or loss of endogenous Bcl-xL

Priscilla N. Kelly[1,2], Andreas Strasser[2] and Jerry M. Adams[2]

[1]Metabolism Branch, CCR, NCI; [2]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Evasion of apoptosis is an accepted prerequisite for the development of most, if not all, cancers, yet the mechanisms that sustain survival of most cancer cells remain unknown. Bcl-2 family members are key apoptosis regulators and include proteins essential for cell survival (e.g. Bcl-2, Bcl-xL and Mcl-1). Over-expression of Bcl-2 or Bcl-xL in transgenic mice promotes tumor development, yet only a minority of human tumors harbor the cytogenetic abnormalities that provoke their over-expression. It is therefore likely that survival signaling through endogenous Bcl-2-like proteins is required to prevent the elimination of nascent neoplastic cells. We have addressed the role of endogenous pro-survival Bcl-2 family proteins in the initiation and development of lymphoma, using the E-myc transgenic mouse model of pre-B/B lymphoma. Bcl-2 loss had no affect on the onset, incidence or severity of lymphoma. In contrast, Bcl-xL proved to be critical for sustaining the survival of cells undergoing malignant transformation, and genetic deletion of Bcl-xL abrogated lymphoma development. Thus, Bcl-xL but not Bcl-2 is critical for opposing Myc-induced apoptosis at a critical early stage of tumorigenesis. A number of compounds that inhibit the activity of Bcl-2-like pro-survival proteins are currently undergoing clinical trials. One such agent that has shown considerable efficacy for the treatment of established human tumors is the BH3 mimetic and Bcl-xL antagonist ABT-263. We therefore tested the pre-clinical version, ABT-737, for its potential to retard tumor development in pre-malignant E-myc mice. Pharmacological blockade of endogenous Bcl-xL by ABT-737 enhanced the apoptosis of pre-leukemic E-myc B cells, and significantly prolonged disease-free survival. These findings identify Bcl-xL as a prerequisite for the emergence of c-Myc-driven pre-B/B lymphoma in mice, and suggest that BH3 mimetic drugs may provide a prophylactic strategy for c-Myc-driven tumors and potentially aid management of patients with hereditary cancer syndromes.

#74

Genes that mediate breast cancer dormancy in the liver and lung

Joji Nakayama, Jean-Claude A. Marshall, and Patricia S. Steeg

Womens Cancers Section, Laboratory of Molecular Pharmacology, CCR, NCI, Bethesda

Nm23 is one of metastasis suppressor genes, significantly reduce metastasis progression without effect on primary tumor when it is overexpressed. Previous studies identified a G-protein coupled receptor for the bioactive lipid lysophophatidic acid (LPA1) as a being inversely correlated with Nm23. LPA1 inhibitor (Debio 0719) treatment significantly decreased metastasis progression in two model systems of breast cancer, the murine mammary cancer cell line 4T1 and human breast cancer cell line MDA-MB-231. Moreover, these studies also have shown 0719 treatment induced metastatic cell dormancy in these two models. We hypothesis this site specific induced dormancy indicates a microenvironmental role in effects of LPA1 inhibition in vivo. To assess this we analyzed the effects of 0719 treatment on potential p38 activating cell surface receptors. Quantitative RT-PCR showed an increased of 4-fold in PDGFRα, 2-fold in IGF-1Rβ and 1.8-fold in TNFR1 expression in 4T1 cells treated with 0719. This increase was validated by flow cytometry showing a 29.2%, 32.6% and 34.2% increase in cell surface expression of these receptors, respectively. Immunoflourescent staining of liver and lung metastases from mice treated with 0719 revealed increased staining for PDGFRα, IGF-1Rβ and TNFR1 compared to vehicle control mice. The effects of 0719 on the elevation of phospho-p38 in vivo was recapitulated in vitro by the addition of recombinant PDGFAA and BB, IGF1 and TNFα with 0719 on 4T1 cells. These results indicate that a microenvironmental stimulus is necessary for the induction of dormancy in metastasis by an LPA1 inhibitor. Furthermore, treatment with 0719 induced an increase in expression of these cell surface receptors for cytokines specific to the lung and liver microenvironment.

#75

Decreased Pluripotency and Limited Lineage Commitment in CASZ1 Haploinsufficient Murine Embryonic Stem Cells

Stanley He, Ryan Virden, Zhihui Liu, and Carol J. Thiele

Pediatric Oncology Branch, CCR, NCI

Background: Tumor suppressor CASZ1(1p36.22) is often lost in poor prognosis neuroblastomas (NB) with an undifferentiated histopathology. Differentiation of NB cells upon CASZ1 restoration indicates CASZ1 loss may contribute to the undifferentiated phenotype during NB tumorigenesis. How CASZ1 functions during normal mammalian development is unknown. Using the murine embryonic stem cell (mESC) in vitro embryogenesis model, we investigated the effects of CASZ1 haploinsufficiency and CASZ1 reconstitution on mESC pluripotency and differentiation programs. Methods: Differentiation in mESCs, wild-type (WT) and Casz1 gene-trap (CAS+/-mESCs), was evaluated using mESC adipogenesis, cardiogenesis, and neurogenesis assays. For rescue experiments, CAS+/-mESCs were transfected with a bacterial artificial chromosome containing the full human CASZ1 genomic locus (ESC-BAC-CASZ1). Gene expression was evaluated by qPCR and western blotting. Results: Analysis of CAS+/-mESCs revealed elevated N-MYC protein (>5.5 fold) and higher c-Myc mRNA (~5.5 fold), but lower Oct4 (0.8 fold) and Nanog (0.8 fold) expression compared to WT mESCs. CAS+/-mESCs maintain adipogenesis potential shown by Oil-Red-O positive staining for adipocytes, but cardiogenesis potential is lost as CAS+/-mESCs are unable to form beating embryoid bodies and neurogenesis potential is lost as evidenced by a failure to extend -III tubulin positive neuritic processes. However, in the ESC-BAC-CASZ1 there is normalization of CASZ1 mRNA (1-2 fold) and protein (0.6-1.2 fold) levels, a 3.8 fold decrease in N-MYC protein levels, and a 2 fold decrease in c-Myc mRNA relative to CAS+/-mESCs. Moreover, Oct4 (1.3 fold) and Nanog (1.1 fold) expression normalize compared to WT mESCs. ESC-BAC-CASZ1 also demonstrated full neurogenesis potential, but limited cardiogenesis potential. Conclusions: The data shows CASZ1 haploinsufficent mESCs exhibit multipotency, but CASZ1 restoration can rescue neurogenesis potential. We propose CASZ1 haploinsufficiency promotes trophoectoderm differentiation, thereby limiting further differentiation into cardiomyocytes or neurons. Compellingly, CASZ1 restoration can rescue neurogenesis potential and may explain the undifferentiated phenotype seen in many poor prognosis NBs.

#76

Breast cancer cells expressing Nanog have characteristics of cancer stem cells

Marcella A. Kaddoura[1], Alan T. Brooks[2], and Thomas J. Sayers[2]

[1]Cancer Inflammation Program, NCI-Frederick, MD; [2]SAIC-Frederick Inc. Frederick, MD

Introduction: Many tumors have been demonstrated to contain a subpopulation of cancer stem cells (CSC) whose epigenetic modifications convey resistance to conventional therapies. Furthermore, tumor expression of genes associated with pluripotent embryonic cells, such as Nanog, often correlates with poor prognosis in cancer patients. We evaluated Nanog expression in 4T1.2 murine breast carcinoma cells, as a biomarker for aggressive CSC populations. Methods: Introduction of a Nanog promoter reporter that drives destabilized GFP into 4T1.2 cells resulted in a stable expression of GFP in about 1% of the cells. These GFP+ and GFP- populations were obtained by fluorescence activated cell sorting (FACS) after which many of the GFP+ cells reverted to a GFP- phenotype. To derive and maintain a stable phenotype, single cell clones of these GFP+ and GFP- cells were isolated. To test for invasion and migration capacity, a Matrigel Invasion Chamber assay was used in which cells were added to the top of the gel and allowed 24 hours to invade. The cells were then fixed, stained and counted. The MTS assay was used in studying growth and drug sensitivity of the GFP+ and GFP- clone populations. Results: On comparing a GFP+ and GFP- clone, both clones grew equally well in media supplemented with 5% fetal bovine serum, whereas the GFP+ clone grew significantly more slowly in minimal media supplemented with growth factors. The GFP+ clone formed more numerous and larger spheroid colonies following growth in soft agar, and demonstrated an increased invasive capacity in vitro. Furthermore, the GFP+ clone revealed increased sensitivity to certain drugs in the MTS assay. Discussion: These properties are consistent with the GFP+ clone having a CSC phenotype and suggest that Nanog promoter activity is a robust marker for 4T1.2 CSCs, allowing an easy isolation and characterization of these cells based on GFP expression.

#77

Transcriptomic Profiling Reveals Hepatic Stem-like Gene Signatures and Interplay of miR-200c and EMT in Intrahepatic Cholangiocarcinoma

Naoki Oishi[1], Mia Kumar[1], Stephanie Roessler[1], Junfang Ji[1], Marshonna Forgues[1], Anuradha Budhu[1], Xuelian Zhao[1], Jesper B. Andersen[2], Snorri S. Thorgeirsson[2], Lun-Xiu Qin[3], Zhao-You Tang[3], Taro Yamashita[4], Shuichi Kaneko[4], Yoon Jun Kim[5], Hyun Goo Woo[5], and Xin Wei Wang[1]

[1]Laboratory of Human Carcinogenesis and [2]Laboratory of Experimental Carcinogenesis, CCR, NCI, NIH, Bethesda, MD; [3]Liver Cancer Institute, Fudan University, Shanghai, China; [4]Liver Disease Center and Kanazawa University Hospital, Kanazawa University, Kanazawa, Japan; [5]Lab of Systems Medicine, Department of Physiology, Ajou University School of Medicine, Yeongtong-gu, Suwon, Korea

Intrahepatic cholangiocellular carcinoma (ICC) is the second most common type of primary liver cancer. However, its tumor heterogeneity and molecular characteristics are largely unknown. In this study, we conducted transcriptomic profiling of 23 ICC tumor specimens derived from Asian patients using Affymetrix mRNA microarray and Nanostring microRNA array to search for unique gene signatures linked to tumor subtypes and patient prognosis. We validated the signatures in additional 68 ICC cases derived from Caucasian patients. We found that both mRNA and microRNA expression profiles could independently classify Asian ICC cases into two main subgroups, one of which shared gene expression signatures with previously identified hepatocellular carcinoma (HCC) with stem cell gene expression traits. ICC-specific gene signatures could predict survival in Asian HCC cases and independently in Caucasian ICC cases. Integrative analyses of the ICC-specific mRNA and microRNA expression profiles revealed that a common signaling pathway linking miR-200c signaling to epithelial-mesenchymal transition (EMT) was preferentially activated in ICC with stem cell gene expression traits. Inactivation of miR-200c resulted in an induction of EMT while activation of miR-200c led to a reduction of EMT including a reduced cell migration and invasion in ICC cells. Conclusion: Our results indicate that ICC and HCC share common stem-like molecular characteristics and poor prognosis. We suggest that the specific components of EMT can be exploited as critical biomarkers and clinically relevant therapeutic targets for an aggressive form of stem cell-like ICC.

#78

Aminoglycosides readthrough PTC and increase DNA repair in homozygous and heterozygous xeroderma pigmentosum group C

Christiane Kuschal, John J. DiGiovanna, Sikandar G. Khan, and Kenneth H. Kraemer

DNA repair section, Dermatology Branch, CCR, NCI

Xeroderma pigmentosum (XP) patients suffer from a >10000 fold increased skin cancer risk caused by mutations in nucleotide excision repair (NER) genes. Many XP patients have premature termination codon (PTC) in the XPC gene that inhibits NER. In-vitro studies demonstrated that aminoglycosides like Geneticin and Gentamicin are able to "read through" PTC, leading to partial induction of missing proteins in ataxia-telangiectasia, Hurler syndrome, and cystic fibrosis. We recently showed that levels of Geneticin that reduced cell survival by 50% can partially induce XPC protein expression and DNA repair in 2 XPC-deficient cell lines with homozygous CGA Arg155>X or CGA Arg 220>X PTC mutations. We now investigated the effects of Geneticin in 4 compound heterozygote XPC cell lines containing only 1 PTC. All cells tested lack XPC protein and show no repair of UV-induced 6-4 photoproducts (6-4PP) or cyclobutane pyrimidine dimers (CPD). In Geneticin treated cells containing CGA Arg155>X, CGA Arg415>X, and AAG Lys692>X but not in cells with AAA Lys522>X, XPC protein, and recruitment of XPB and XPD proteins was visualized at sites of UV damage followed by removal of 6-4PP. However, the effect of Geneticin was less efficient than in the Arg>X homozygous cells. Interestingly, unlike in the Arg>X homozygous cells, Geneticin had no effect on the repair of CPD in the compound heterozygotes. In addition, levels of Gentamicin that reduced cell survival by only 20-30% also increased readthrough of 6-4PP and CPD in the Arg>X homozygous cells, but the effect was lower compared with Geneticin. Our results show that aminoglycosides can induce readthrough of homozygous and heterozygous PTC in XPC-deficient cells. This efficiency depends on the specific PTC and type of compound. XPC patients harboring PTC may benefit from topical aminoglycoside treatment in order to prevent skin cancer by partially correcting their DNA repair defect.

#79

The Role of TRADD in Skin Tumorigenesis

Elena Pobezinskaya, Swati Choksi, and Zhenggang Liu

Cell and Cancer Biology Branch, CCR, NCI

Clinical evidence links chronic NFκB-dependent inflammation with epithelial skin tumors. By generating TRADD null mice we demonstrated the importance of TRADD in NFκB and MAP-kinase activation during TNFR1 signaling. Now, we determined the outcome of TRADD deletion on cutaneous inflammation and skin tumor promotion using the established model of two-stage carcinogenesis. In this model, mice are treated once with the carcinogen, DMBA, which initiates the tumor, and 2 weeks later with TPA for 30 weeks. We used mice with a keratinocyte-specific deletion of TRADD enabling us to study keratinocytes in tumorigenesis independent of the microenvironment. Papillomas were counted weekly starting from week 10. Our results demonstrate that KO mice developed significantly less papillomas compared to wild type (WT) mice. Since TNFα is a major cytokine pivotal to cutaneous inflammation and since KO keratinocytes do not respond to TNFα we reasoned that inflammation triggered by TPA treatment may be impaired in the absence of TRADD. Surprisingly, both KO and WT animals responded to TPA with similar epithelial hyperplasia. Moreover, stromal infiltration by immune cells looked unaltered in the absence of TRADD and there was no difference in TNFα and IL-1β production after TPA treatment between the two groups. Altogether these data indicate that inflammation is not affected by the absence of TRADD and is not responsible for the observed reduction in tumor development. Earlier studies have determined that the main target of DMBA is H-Ras gene, activation of which leads to senescence or growth arrest in primary cells. Our preliminary data suggest that KO keratinocytes that carry H-Ras mutation are senescent/arrested due to their inability to respond to TNFα and may be other stimuli as well. These studies will provide more insights into the tumor promoting effect of inflammation on tumor cells leading to the development of novel cancer treatments.

#80

Exposure to nucleoside reverse transcriptase inhibitors (NRTIs) induces genotoxicity persistent for up to 3 years in Erythrocebus patas monkeys

Lorangelly Rivera Torres[1], Miriam C. Poirier[1], Sayeh Gorjifard[1], Yongmin Liu[1], Ruth A. Woodward[2], and Ofelia A. Olivero[1]

[1]CDI Section, LCBG, NCI, NIH, Bethesda, MD; [2]NIH Animal Center, Poolesville, MD

Antiretroviral NRTIs are given during pregnancy and after birth to reduce maternal-fetal HIV-1 transmission. These drugs are DNA replication chain terminators, and induce: centrosomal amplification (CA, >2 centrosomes/nucleus), resulting in abnormal chromosomal segregation; as well as micronuclei (MN), and MN with whole chromosomes (MN+C). Pregnant Erythrocebus patas (patas) monkey dams (n=2-3/group) were given human-equivalent daily doses of the NRTIs Zidovudine (AZT) and Lamivudine (3TC), for the last half (10 wk) of gestation. At birth and 3 years of age, mesenchymal cells were cultured from bone marrow of offspring and examined for CA, MN and MN+C. For the patas taken at birth, CA was found in 2.03 0.8% and 4.50 0.3% of cells from unexposed and AZT/3TC-exposed fetuses, respectively (p =0.035). There were 1.35 0.3% and 2.34 0.4% of cells with MN in unexposed fetuses and AZT/3TC-exposed fetuses, respectively (p = 0.153). MN+C were found in 0.21 0.1% and 0.83 0.1% of cells from unexposed and AZT/3TC-exposed fetuses, respectively (p = 0.009). For the patas taken at 3 years of age, CA was found in 1.131.2% and 5.29% (n=1) of cells from unexposed and AZT/3TC-exposed patas, respectively. Unexposed and AZT/3TC-exposed fetuses had 0.44 0.1% and 3.11 0.2 of cells with MN, respectively (p=0.0017). For MN+C, unexposed and AZT/3TC-exposed fetuses had 0.19 0.01% and 1.09 0.1% of cells affected, respectively (p=0.016). Therefore, genotoxicity, including abnormal chromosomal segregation and aneuploidy, initiated by in utero NRTI exposure, persists in patas bone marrow up to 3 years of age.

Appendix A:
Resources and Core Facilities

#81

Electron Microscopy Laboratory

Ulrich Baxa, Kunio Nagashima, Adam Harned, Christina Burks, Ferri Soheilian, Anne Kamata, and David Parmiter

EML, ATP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD

The Electron Microscopy Laboratory (EML) provides ultrastructural analysis of biological samples and nano-particles using state-of-the art scanning and transmission electron microscopes (SEM and TEM). In collaboration with NCI users, we have developed new image analysis techniques as well as sample preparation procedures that are tailored to the specific needs of each of our customers. The EM Core Facility has three distinct functions: shared service (cost per sample), dedicated service (HIV-Drug Resistance Program and Nanotechnology Characterization Lab (NCL), NCI-Frederick), and new technology development and enhancement. Capabilities of the EML include, but are not limited to: room-temperature processing of biological samples for thin sectioning TEM analysis or for SEM analysis, negative staining analysis, room-temperature electron tomography of sections or nano-particles to determine a three dimensional structure, cryo-TEM of macromolecular structures very close to their native state, elemental analysis using X-ray Energy Dispersive Spectroscopy (EDS), and correlated fluorescence and electron microscopy. Currently we are developing and enhancing several technologies including the correlated fluorescence and electron microscopy and cryo-electron tomography. Cryo-electron tomography would allow visualizing e.g. HIV and SIV particles in 3 dimensions in their solution state. This technique involves computer aided serial imaging over a range of tilt angles, followed by tomographic reconstruction into a 3D image.

#82

Services available from the Protein Expression Laboratory at NCI-Frederick

Dominic Esposito*, Bill Gillette, Butch Hopkins, and Rachel Bagni

Protein Expression Laboratory, Advanced Technology Program, SAIC-Frederick, Inc.

The Protein Expression Laboratory (PEL) can assist you in your research needs for cloning, protein production, virus preparation, and molecular detection. Our Clone Optimization group can assist you in construction of state-of-the-art expression clones utilizing the latest combinatorial recombination-based cloning methods. The Eukaryotic Expression Group and Protein Purification Group can take protein expression constructs and express them in any host, utilizing microscale purification to screen for the best candidates rapidly and inexpensively prior to scaleup. We can generate proteins in any quantity from micrograms to grams, and have various methods for QC of proteins depending on the needs of the investigator. Our Viral Technology Group packages and titers various viruses including lentivirus, adenovirus, retrovirus, and adeno-associated virus (AAV). The Molecular Detection Group carries out cell line assays including mycoplasma and pathogen detection, cell line identity (STR) analysis, and virus detection, as well as develops ELISA-based assays using multiplex technology. Taken together, the services offered by the PEL can enhance your research in many ways, and we are always eager to collaborate on complicated projects that cross the groups within the laboratory. In addition, we can interface with other Advanced Technology Program laboratories on larger scale systems biology projects.

#83

The CCR Confocal Microscopy Core provides new dimensions in imaging

Susan H. Garfield, Poonam Mannan, and Langston Lim

Laboratory of Experimental Carcinogenesis, CCR, NCI

The CCR Confocal Core provides state-of-the-art microscopic analyses to better understand critical biological structures and cellular processes involved in cancer. Confocal Microscopy is a valuable research tool for imaging fluorescently labeled specimens, permitting accurate, non-invasive optical sectioning for 3D tomography or time-lapse studies. Techniques used in this facility are: 1) co-localization of FP fusion proteins with organelles (mitochondria, endoplasmic reticulum, lysosomes, Golgi, and endosomes), 2) labeling of fixed cells to demonstrate membrane ruffling, cytoskeletal organization, focal adhesions and other cell morphology, 3) time-lapse of translocation in living cells of FP fusion proteins, 4) fluorescent indicators of oxidative stress in live cells, 5) 4D imaging during cell division, 6) photoactivation of fluorescent proteins and 7) Second Harmonic Generation imaging (SHG) of whole live tissue/organ. Both Zeiss LSM 510 Confocal systems are suitable for Fluorescent Recovery After Photobleaching (FRAP) and Fluorescent Resonance Energy Transfer (FRET) experiments. The Zeiss LSM 510 NLO with Meta detector, for separating fluorophores with close emission spectra, was added in 2004. This 2-photon system allows imaging thick tissue with minimum tissue damage. An x,y scanning stage and environmental chamber were added later. The Zeiss 710 with 2-photon laser provides 1p and 2p Fluorescence Lifetime Imaging (FLIM). It has an x,y scanning stage for tiling and an environmental chamber plus non-descanned detectors (NDDs) for deeper imaging, and 32 channel PMT for spectral imaging. Specialized software and computers are also available to users. The instrumentation, organization and services of the CCR Confocal Core Facility will be presented with examples of research projects from some of the 400+ investigators who have utilized these resources.

#84

The DNA Sequencing and Digital Gene Expression Core

Kathleen Hartman[1], Patricia Johnson, Steve Shema, and Snorri Thorgeirsson

[1]LEC, CCR, NCI

The DNA Sequencing Facility provides investigators in the NCI, with rapid processing of their DNA sequence samples. Our goal is to generate accurate results as rapidly and efficiently as possible. Last year over 80,000 samples were processed. 99% of the samples are returned within one business day. We charge $2.00 for electrophoresis and $7.00 for the sequencing reactions plus electrophoresis. Bulk rates prices are available. The Core can also provide ABI Big Dye Version 1.1 at cost. The Core offers digital Gene Expression using the NanoStrings nCounter Analysis System. This system is a fully automated platform for the next generation of digital gene expression analysis. This unique technology is completely non-enzymatic and utilizes fluorescent barcodes hybridized to individual mRNA molecules to directly count the relative abundance of each transcript of interest in a sample. The assay is performed directly on total RNA (no RT, IVT, and PCR) or lysates, from a wide variety of sample types including cells, tissues, FFPE, and blood. It enables multiplexed profiling of 20 to 500+ genes. The platform is ideal for miRNA profiling, pathway expression profiling, validation of complex gene expression signature and high throughput RNA-Seq and array validation and CNV analysis. In the spring of 2012 the core will begin to offer Next Gen Sequencing services. We are in the process of acquiring Illumina MiSeq sequencer. MiSeq is recommended for relatively small Next Gen Sequencing applications like amplicon, small RNA and small genome sequenceing, ChiPSeq, etc. For the past five years, the MiniCore has provided a location for an Agilent Microarray Scanner. The Core staff controls the calendar sign up as well as providing bench space for sample preparation, the oven and the scanner. In 2008 we added a Stratagene Real Time PCR Mx3005P. Anyone can sign up and use this instrument.

#85

Optical Microscopy and Analysis Laboratory

Stephen Lockett, Prabhakar Gudla, Kaustav Nandy, Tommy Turbyville, Kimberly Peifley, De Chen, and Alla Brafman

Advanced Technology Program, NCI-Frederick

The lab provides advanced optical microscopy image acquisition and analysis that includes confocal, photo-activation localization microscopy (PALM) and single particle tracking, undertakes collaborative research and performs technology development. Collaborative research examples include: With the CCR labs of Dr. John Beutler and Dr. Karlyne Reilly and supported by an external contract, we are studying how the cell cytoskeleton dynamically participates in the maintenance and development of cell morphology, intracellular trafficking, cell division, and cell movement. Given the direct connection between actin and cell motility, we developed software to quantify cell migration and cell orientation in the gap-closing assay. We began a major endeavor to analyze actin patterns in cell constrained to defined micropatterns, which includes generative modeling to computationally simulate cell patterns. Such micropatterns make analysis of individual cells facile and are revealing subtler changes in cell morphology at low candidate drug doses, which were missed by standard cell culture. In collaboration with Dr. Tom Misteli (CCR), software for automatic gene localization in cell nuclei has been improved in terms of accuracy and for higher throughput analysis. The software has been distributed to several other investigators. Responding to significant increases in biological fluorescence microscopy and to rapidly diversifying technology advancements, the lab is increasingly coordinating its research and technology development activities with other CCR microscopy laboratories, different NIH institutes, and regional universities and national laboratories.

#86

Innovative Sequencing Resources in the CCR Sequencing Facility: Current and Future Applications Using the Illumina and Pacific Biosciences Platforms

Kristie Jones[1], Castle Raley[1], Jyoti Shetty[1], Yongmei Zhao[2], Ayobola Akingbade[1], Jessica Dickens[1], Yuliya Kriga[1], Yelena Levin[1], Shashikala Ratnayake[2], Julie Scuffins[2], Jennifer Troyer[3], Emir Khatipov[2], Qiang Sun[2], Alexander Levitsky[2], Robert Stephens[2], Belynda Hicks[3], David Munroe[4], Jack Collins[2], Michael W. Smith[3], Paul Meltzer[5], and Bao Tran[1]

[1]Sequencing Facility, Advanced Technology Program, SAIC-Frederick, Inc., NCI- Frederick, Gaithersburg, MD; [2]Advanced Biomedical Computing Center, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [3]Genetics and Genomics Group, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [4]Technology Development, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD; [5]Genetics Branch, National Cancer Institute, Bethesda, MD

The CCR-SF is a second and third generation high-throughput sequencing core laboratory established by the Center for Cancer Research (CCR). The SF is located at the Advanced Technology Center (ATC) in Gaithersburg, MD, and offers sequencing services on both the Illumina and Pacific Biosciences platforms. These two platforms have complementary strengths and can be used separately or in a combined approach to answer many genomics questions. The established Illumina platform (the SF has two HiSeq 2000s and two GAIIx sequencers), has been in production at the SF since 2009. The newer Pacific Biosciences platform, the PacBio RS, was acquired by the SF prior to its commercial release as a part of their Pioneer program, giving us an advantage in developing expertise in this platform early in its development. Both offer unique advantages for different sequencing applications, including whole genome sequncing, exome and transcriptome sequencing, targeted amplicon resequencing, ChIP-seq, base modification detection, and sequencing complex repeats, secondary structures, and AT and GC-rich sections of DNA. Here we will discuss the current applications these platforms can offer, as well as those applications under development.

#87

Applied Genomics Services at the Laboratory of Molecular Technology

Nina Bubunenko, Scott Coccodrelli, Viktoriya Grinberg, Todd Hartley, Jason Mitchell, Kristen Pike, Teri Plona, Castle Raley, Arati Raziuddin, Nicole Shraeder, Michael Smith, Dan Soppet, Myla Spencer, Claudia Stewart, Robin Stewart, Ling Su, David Sun, Jennifer Troyer, and Xiaolin Wu

Laboratory of Molecular Technology, Advanced Technology Program, SAIC-Frederick, Inc., Frederick, Md

The Laboratory of Molecular Technology (LMT) is an open core, fee for service, facility providing an extensive list of applied genomics technologies for NCI investigators. The LMT offers support in microarray technologies on both the Agilent and Affymetrix array platforms. Gene expression and miRNA expression on the Nanostring single molecule system as well as qPCR and single cell qPCR on the Fluidigm Microfluidics platform. Sequencing support is currently provided on either the Roche/454 Next generation sequencers or for Sanger sequencing on the ABI-3730. We also provide CLIA certified sequencing for support of clinical mutation detection. We will be offering an extensive set of additional sequencing options this year including short read sequencing on the Life Technologies 5500xl, The Ion Torrent PGM and the Pacific Biosciences RS. We provide a range of library preparation services including whole exome capture using Agilent SureSelect and amplicon development using the Fluidigm access array. The LMT works with partners in the Advanced Technologies program, commercial environment and within the NCI to identify, test, and implement the newest technologies to advance your research. The LMT can provide guidance on the strengths and weaknesses of several applied genomics technologies and we are happy to discuss options to make your research successful.

#88

The Protein Chemistry Laboratory at NCI-Frederick

Lakshman Bindu, Young Kim, Karen Worthy, Simona Colantonio, Oleg Chertov, and Andrew Stephen

Protein Chemistry Laboratory, Advance Technology Program, SAIC-Frederick, NCI-Frederick, Frederick, MD

The Protein Chemistry Laboratory in the Advanced Technology Program at NCI-Frederick offers unparalleled capabilities in surface plasmon resonance (SPR) spectroscopy and fluorescence approaches for characterization of molecular interactions, and maintains complementary advanced protein chemistry techniques for protein identification including mass spectrometry (MS) and Edman sequencing. PCL has state-of-the art matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) MS with MALDI-imaging capabilities and a MALDI-TOF equipped with ultra-high mass detection. PCL also provides high-performance liquid chromatography (HPLC) purification and quality control of proteins and oligonucleotides. The objective of the laboratory is to work collaboratively with colleagues and help them achieve their research objectives.

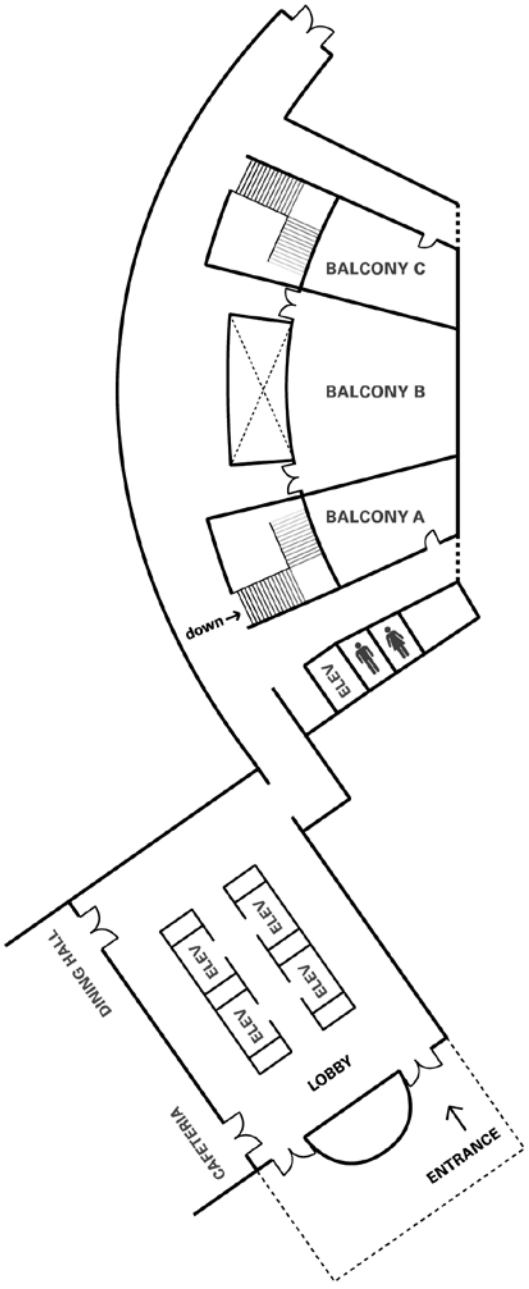
Appendix B:

Author Index

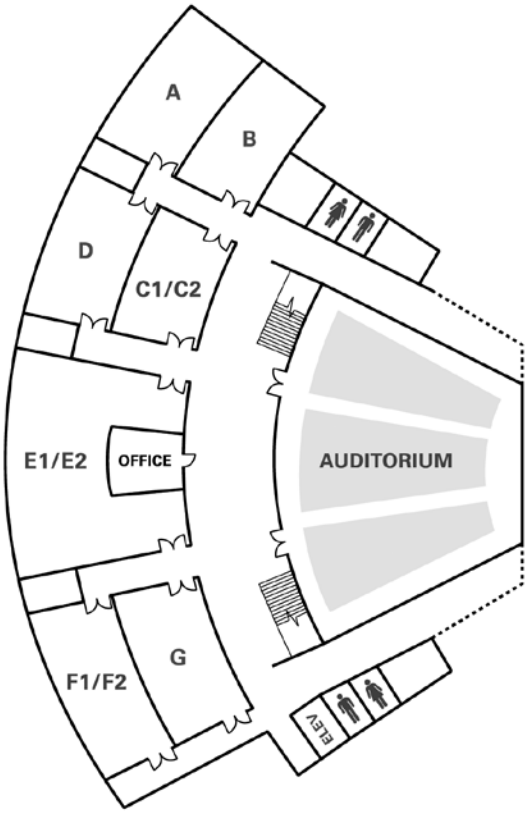
Appendix C:

Facility Map and CCR-FYI Flyer

NATCHER CONFERENCE CENTER
LEVEL 1 (MAIN LEVEL)



NATCHER CONFERENCE CENTER
LOWER LEVEL



NCI Center for Cancer Research

NATIONAL CANCER INSTITUTE

FELLOWS & YOUNG INVESTIGATORS

CCR FYI

What is the CCR-FYI?

The NCI CCR Fellows and Young Investigators (FYI) Association was organized to foster the professional advancement of young scientists at the CCR and is supported by the NCI Office of Training and Education (OTE).

Who can participate?

All young investigators including postdocs, postbacs, graduate students, research fellows, clinical fellows, technicians, and staff scientists.

What opportunities does FYI offer?

Frederick Postdoc Seminar Series – Alternating Wednesdays at 1:30pm in Bldg 549 Auditorium
Bethesda PASS (Presentation and Seminar Skills) – Monthly series to give tutoring assistance for presentations
Quarterly newsletter – We are always looking for volunteers to write articles
Organizing the annual Fellows Colloquium
Outreach – Get involved with the community both on and off campus
Assisting in orientation for new fellows
Social events
Annual survey – Evaluating the demographics and opinions of fellows in the NCI CCR
Serving as a liaison to administration programs
Enhancing intramural training program – promote communication between mentors and trainees

GET INVOLVED!

Attend our monthly Steering Committee meetings
The last Thursday of the month at 11:00am
Bldg 549 in Frederick, Bldg 40 in Bethesda

Sign up for the listserv
Email nciccrfyi@mail.nih.gov

Attend our Annual Colloquium
Held in the spring
Present your work and be eligible to win a travel award
Network with your fellow trainees
Attend skill building workshops
Talk to employers at the career fair
Get inspired from outstanding keynote speakers

FYI is an excellent way to network and build leadership skills!

Supported by the CCR Office of Training and Education and the CCR Office of the Director
http://ccr.cancer.gov/careers/fellows

Appendix D:
Notes