

Susan G. Komen for the Cure Research Grants – Fiscal Year 2009

This slate of research grants was approved by Komen's national board of directors on March 25, 2009. These grants will be funded upon the execution of grant agreements between Komen and the grantee institutions.

Promise Grants

This exciting award mechanism provides substantial funding for multidisciplinary teams of clinical and laboratory investigators to conduct a set of related studies addressing an overarching issue of critical importance in breast cancer. *Funding: Up to \$7.5M (no more than \$1.5M per year) for up to 5 years.*

[Targeting Death Receptors for the Treatment of Triple Negative Breast Cancer](#)

Co-Investigators:

Andres Forero, MD

Tong Zhou, MD

University of Alabama at Birmingham

Birmingham, AL

Scientific Area: Targeted Therapies

\$6,420,821

[Comprehensive Biomarker Discovery Project for Bevacizumab in Breast Cancer](#)

Co-Investigators:

Bryan Schneider, MD

David Flockhart, MD, PhD

Indiana University, Indianapolis

Indianapolis, IN

Scientific Area: Diagnostic and Prognostic Biomarkers

\$5,825,618

[Therapy-relevant Stratification of Breast Cancer Patients: Integrating Pathology and Biomarker Analyses](#)

Co-Investigators:

Hallgeir Rui, MD, PhD

Edith Mitchell, MD

Thomas Jefferson University

Philadelphia, PA

Scientific Area: Targeted Therapies

\$6,676,115

Pending Execution of Grant Agreements

[Development of novel early detection and prevention strategies for ER negative breast cancer](#)

Co-Investigators:

Dihua Yu, MD, PhD

M.D. Anderson Cancer Center, University of Texas

Houston, TX

Victoria Seewaldt, MD, PhD

Duke University

Durham, NC

Scientific Area: Diagnostic and Prognostic Biomarkers

\$6,750,000

Investigator Initiated Research

These grants seek to stimulate exploration of important issues and novel approaches that will lead to reductions in breast cancer incidence and/or mortality within the next decade.

Funding: \$200,000 per year for 2 to 3 years.

[Inhibition of Breast Cancer Cell Aggressiveness by Cannabidiol](#)

Sean McAllister, PhD

Co-PI: Pierre-Yves Desprez, PhD

California Pacific Medical Center

San Francisco, CA

Scientific Area: Targeted Therapies

\$593,713

[Breast Cancer Risk Reduction in Primary Care Clinics: A Bilingual Intervention for Women and Physicians](#)

Celia Kaplan, DrPH

University of California at San Francisco

San Francisco, CA

Scientific Area: Nutritional, Behavioral and Lifestyle Prevention

\$599,803

[From Bench to Bedside: Treatment of Breast Cancer Brain Metastasis with ¹³¹I and Radiosensitizers](#)

Irene Wapnir, MD

Co-PI: Michael Goris, PhD

Stanford University, School of Medicine

Stanford, CA

Scientific Area: Nonsystemic Therapies

\$600,000

[Induction of apoptosis of highly invasive breast cancer cells by novel small molecule inhibitors of intracellular urokinase plasminogen activator](#)

Kermit Carraway, PhD

Co-PI: Fredric Gorin, MD, PhD

University of California at Davis, School of Medicine

Davis, CA

Scientific Area: Targeted Therapies

\$600,000

Pending Execution of Grant Agreements

[Restoration of MicroRNA-200c: a Novel Differentiation Therapy for Treatment of Aggressive Breast Cancers](#)

Jennifer Richer, PhD
University of Colorado Health Sciences Center Aurora, CO
Scientific Area: Targeted Therapies
\$600,000

[Targeting the pro-inflammatory milieu of the involuting gland to suppress pregnancy-associated breast cancer metastasis](#)

Pepper Schedin, PhD
Co-PI: Virginia Borges, MD
University of Colorado Health Sciences Center Aurora, CO
Scientific Area: Pathobiology
\$599,696

[In Situ Detection of Cancer Stem Cells to Predict Non-Nodal Metastasis in Breast Cancer](#)

David Rimm, MD, PhD
Yale University New Haven, CT
Scientific Area: Diagnostic and Prognostic Biomarkers
\$600,000

[Cell Death Signaling in Endocrine Responsiveness](#)

Robert Clarke, PhD
Co-PI: Bassem Haddad, MD
Georgetown University, Lombardi Comprehensive Cancer Center Washington, DC
Scientific Area: Endocrine Therapies
\$599,123

[Development of Agonistic 4-1BB Aptamers to Enhance Vaccine-Induced Tumor Immunity](#)

Eli Gilboa, PhD
University of Miami, School of Medicine Miami, FL
Scientific Area: Immunotherapies
\$600,000

[Predicting Breast Cancer by Detection of Promoter Hypermethylation in Serum DNA](#)

Susan Sturgeon, DrPH
University of Massachusetts Amherst, MA
Scientific Area: Diagnostic and Prognostic Biomarkers
\$236,171

[Functional consequence of deregulated HoxB9 expression in breast tumors](#)

Shyamala Maheswaran, PhD
Massachusetts General Hospital Charlestown, MA
Scientific Area: Diagnostic and Prognostic Biomarkers
\$600,000

Pending Execution of Grant Agreements

[Systematic RNAi Knockdown and Systems Biology Approach Reveal Roles and Interactions of Signaling Pathways in Mechanisms of Acquired Antiestrogen Resistance](#)

Toshihiro Shioda, MD, PhD
Massachusetts General Hospital
Scientific Area: Endocrine Therapies
\$599,995
Charlestown, MA

[Functional Metabolic Near-infrared Tomographic Optical Breast Imaging \(TOBI\) to Monitor Response to Neoadjuvant Therapy in Breast Cancer](#)

Steven Isakoff, MD, PhD
Massachusetts General Hospital
Scientific Area: Diagnostic and Prognostic Biomarkers
\$599,842
Boston, MA

[Plasma marker of oxidative stress and risk of breast cancer: a prospective study](#)

Heather Eliassen, ScD
Brigham and Women's Hospital
Scientific Area: Epidemiology and Risk Assessment
\$587,903
Boston, MA

[Lysosomal Associated Protein Transmembrane 4B \(LAPTM4B\), a novel drug resistance gene in breast cancer](#)

Andrea Richardson, MD, PhD
Co-PI: Zhigang Wang, MD, PhD
Dana-Farber Cancer Institute
Scientific Area: Targeted Therapies
\$600,000
Boston, MA

[Genetic variants and gene-environment interactions in relation to breast cancer incidence in African-American women](#)

Julie Palmer, ScD
Trustees of Boston University, BUMC
Scientific Area: Epidemiology and Risk Assessment
\$598,000
Boston, MA

[Evaluation of Low-dose Molecular Breast Imaging as a Screening Tool in Women with Mammographically Dense Breasts and Increased Risk of Breast Cancer](#)

Deborah Rhodes, MD
Mayo Clinic and Foundation, Rochester
Scientific Area: Diagnostic and Prognostic Biomarkers
\$599,558
Rochester, MN

Pending Execution of Grant Agreements

[A Phase I/II Trial of Short Course Pre-Operative Ritonavir in Breast Cancer](#)

David Potter, MD, PhD
University of Minnesota at Twin Cities
Scientific Area: Targeted Therapies
\$600,000
Minneapolis, MN

[Whole Genome Profiling and Functional Genomics in Breast Cancer](#)

Matthew Ellis, MD, PhD
Washington University in St. Louis, School of Medicine
Scientific Area: Diagnostic and Prognostic Biomarkers
\$600,000
St Louis, MO

[TbetaRIII/sTbetaRIII in the Diagnosis and Treatment of Breast Cancer](#)

Gerard Blobe, MD, PhD
Duke University, School of Medicine
Scientific Area: Diagnostic and Prognostic Biomarkers
\$600,000
Durham, NC

[Mammary epithelial cell types as determinant of hypoxia responses in breast cancers](#)

Jen-Tsan Chi, MD, PhD
Co-PI: Jeffrey Marks, PhD
Duke University Medical Center
Scientific Area: Pathobiology
\$600,000
Durham, NC

[Development of Molecularly Targeted Therapeutics for Osteolytic Bone Metastasis](#)

Rakesh Singh, PhD
Co-PI: Dong Wang, PhD
University of Nebraska Medical Center, Eppley Cancer Center
Scientific Area: Targeted Therapies
\$600,000
Omaha, NE

[Analysis of NR1D1, a circadian rhythm metabolic regulator required for breast cancer cell survival](#)

Douglas Conklin, PhD
State University of New York at Albany
Scientific Area: Targeted Therapies
\$246,800
Rensselaer, NY

[Determining Role of EMT in Metastatic Progression of Human Breast Cancer.](#)

Jihe Zhao, MD, PhD
Albany Medical College
Scientific Area: Pathobiology
\$600,000
Albany, NY

Pending Execution of Grant Agreements

[Role of CAV1 in Suppressing Estrogen-Hypersensitivity, DCIS, and Breast Cancer Progression](#)

Michael Lisanti, MD, PhD
Co-PI: Federica Sotgia, PhD
Thomas Jefferson University Philadelphia, PA
Scientific Area: Endocrine Therapies
\$480,000

[Sphingosine Kinase Inhibitors as Anti-Breast Cancer Therapeutic Agents](#)

Jong Yun, PhD
Pennsylvania State University, College of Medicine Hershey, PA
Scientific Area: Targeted Therapies
\$400,000

[P-Rex1 in ErbB Signaling and Breast Cancer](#)

Marcelo Kazanietz, PhD
University of Pennsylvania, School of Medicine Philadelphia, PA
Scientific Area: Diagnostic and Prognostic Biomarkers
\$600,000

[Real Time Assessment of Self-Reporting Chemotherapeutics for Targeted Treatment of Metastatic Breast Cancer](#)

James McIntyre, PhD
Co-PI: Darryl Bornhop, PhD
Vanderbilt University, School of Medicine Nashville, TN
Scientific Area: Localized Chemotherapies
\$600,000

[Role of PELP1 in local estrogen synthesis and breast cancer progression](#)

Ratna Vadlamudi, PhD
Co-PI: Rajeshwar Tekmal, PhD
University of Texas Health Science Center San Antonio, TX
Scientific Area: Endocrine Therapies
\$600,000

[Improving Breast Cancer Outcomes in Older Women through More Effective Use of Screening Mammography](#)

Pamela Vacek, PhD
University of Vermont Burlington, VT
Scientific Area: Nutritional, Behavioral and Lifestyle Prevention
\$382,262

Pending Execution of Grant Agreements

[Crosstalks among Stat3, PR and ErbB-2: a novel biomarker and tool for a new therapy against breast cancer in Latin American women](#)

Patricia Elizalde, PhD
Institute of Biology and Experimental Medicine
Scientific Area: Pathobiology
\$600,000

Buenos Aires, Argentina

Career Catalyst Research

This award mechanism provides unique opportunities for scientists in the early stages of their career to further their research independence by providing support for research exploring important issues and novel approaches that will lead to substantial progress in breast cancer research and reductions in breast cancer incidence and/or mortality within the next decade. *Funding: \$300,000 for 2 years and a performance-based option for a \$150,000 3rd year.*

[Nucleotide variation in the prolactin receptor and its agonists and breast cancer risk](#)

Shehnaz Hussain, PhD
University of California at Los Angeles
Scientific Area: Epidemiology and Risk Assessment
\$299,912

Los Angeles, CA

[Preclinical Fluorescence Lifetime Imaging System For Early Detection of Breast Tumors In vivo](#)

V. Krishnan Ramanujan, PhD
Cedars Sinai Medical Center
Scientific Area: Diagnostic and Prognostic Biomarkers
\$299,919

Los Angeles, CA

[Regulation of ERGgamma in endocrine resistant breast cancer by the ERK pathway](#)

Rebecca Riggins, PhD
Georgetown University
Scientific Area: Endocrine Therapies
\$449,112

Washington, DC

[Targeting Myeloid Derived Suppressor Cells and Regulatory T Cell to Improve the Efficacy of Anti-tumor Vaccine in a Spontaneous Model of Mammary Carcinoma](#)

Paolo Serafini, PhD
University of Miami, School of Medicine
Scientific Area: Immunotherapies
\$450,000

Miami, FL

Pending Execution of Grant Agreements

[Nanocarrier-based Targeted Chemotherapy for Improved Drug Delivery in Locally Advanced Breast Cancer](#)

Mark Cohen, MD
University of Kansas Medical Center
Scientific Area: Nonsystemic Therapies
\$450,000
Kansas City, KS

[Physiologic modeling of common PI3-kinase pathway mutations in human breast epithelial cells to develop mutant-specific targeted therapies](#)

Josh Lauring, MD, PhD
Johns Hopkins University, Kimmel Cancer Center
Scientific Area: Targeted Therapies
\$450,000
Baltimore, MD

[Developing Non-Invasive, in vivo Biomarkers to Guide Antiangiogenic Therapy in Breast Cancer using MRI](#)

Arvind Pathak, PhD
Johns Hopkins University, School of Medicine
Scientific Area: Diagnostic and Prognostic Biomarkers
\$450,000
Baltimore, MD

[Proteomic Identification and Reversal of Mechanisms of Herceptin Resistance in Her2/neu Positive Breast Cancer](#)

Ron Bose, MD, PhD
Washington University in St. Louis, School of Medicine
Scientific Area: Targeted Therapies
\$450,000
St. Louis, MO

[An Integrated Genomic Analysis of the Invasive Breast Cancer Risk Associated with Lobular Carcinoma in Situ](#)

Tari King, MD
Memorial Sloan-Kettering Cancer Center
Scientific Area: Diagnostic and Prognostic Biomarkers
\$299,999
New York, NY

[Breast Adipose Bioactivates Vitamin D and Contributes to Cancer Prevention](#)

Glendon Zinser, PhD
University of Cincinnati
Scientific Area: Pathobiology
\$381,250
Cincinnati, OH

Pending Execution of Grant Agreements

[Autoantibody Profiling As a Novel Method for Early and Personalized Diagnosis for Breast Cancer](#)

Kristi Eglund, PhD
Sanford Research, University of South Dakota
Scientific Area: Diagnostic and Prognostic Biomarkers
\$450,000
Sioux Falls, SD

[A Functional Genomic Approach to Discovery of Breast Cancer Therapeutic Targets](#)

Thomas Westbrook, PhD
Baylor College of Medicine
Scientific Area: Pathobiology
\$450,000
Houston, TX

[DNA Damage as a Biomarker of Risk of Breast Cancer](#)

Isabelle Bedrosian, MD
M.D. Anderson Cancer Center, University of Texas
Scientific Area: Diagnostic and Prognostic Biomarkers
\$296,966
Houston, TX

[Sphingosine Kinase 1 as a New Target for Systemic Therapy against Breast Cancer – Development and Characterization of Novel Sphingosine Kinase 1 Inhibitor](#)

Kazuaki Takabe, MD, PhD
Virginia Commonwealth University
Scientific Area: Targeted Therapies
\$449,556
Richmond, VA

Career Catalyst in Disparities Research

This mechanism, new in FY09, provides a unique opportunity for scientists in the early stages of their career, particularly scientists from populations affected by disparities in breast cancer outcomes, to achieve research independence. *Funding: \$300,000 for 2 years and a performance-based option for a \$150,000 3rd year.*

[Breast Cancer in African American Women: DNA Methylation Studies in Basal-like, HER2+, and Luminal A and B Subtypes](#)

Theresa Swift-Scanlan, PhD
University of North Carolina at Chapel Hill
Scientific Area: Diagnostic and Prognostic Biomarkers
\$450,000
Chapel Hill, NC

[Development of Proteomic Signatures of Risk to Estrogen-Independent Breast Cancer](#)

Catherine Drendall, PhD
Duke University Medical Center
Scientific Area: Chemoprevention
\$450,000
Durham, NC

Pending Execution of Grant Agreements

[Search for novel breast cancer susceptibility genes in pedigrees of African ancestry](#)

Heather Ochs-Balcom, PhD
State University of New York
Scientific Area: Epidemiology and Risk Assessment
\$431,395
Buffalo, NY

[Ethnic differences in the mutational status of the PI3K pathway and breast cancer outcome](#)

Abenaa Brewster, MD
M.D. Anderson Cancer Center, University of Texas
Scientific Area: Diagnostic and Prognostic Biomarkers
\$444,043
Houston, TX

[Chemotherapy Resistance in Hispanic and African American Patients](#)

Ana Gonzalez-Angulo, MD
M.D. Anderson Cancer Center, University of Texas
Scientific Area: Targeted Therapies
\$446,850
Houston, TX

Postdoctoral Fellowship

This continuing mechanism supports training for investigators early in their career and seeks to attract new scientists into careers in breast cancer research. *Funding: \$60,000 per year for 2 years and a performance-based option for a \$60,000 3rd year.*

PDF—Basic Research

[Determining the significance of the AKT\(E17K\) mutation in human breast cancer](#)

Mentor: John Carpten, PhD
Fellow: Bodour Salhia, PhD
Translational Genomics Research Institute (TGen)
Scientific Area: Pathobiology
\$180,000
Phoenix, AZ

[Noncoding RNA, Polycomb and Breast Cancer Progression](#)

Mentor: Howard Chang, MD, PhD
Fellow: Miao-Chih Tsai, PhD
Stanford University, School of Medicine
Scientific Area: Diagnostic and Prognostic Biomarkers
\$180,000
Stanford, CA

[Targeting Therapy-Resistant Tumor Vessels and Preventing Tumor Recurrence](#)

Mentor: Erkki Ruoslahti, PhD
Fellow: Lise Roth, PhD
Burnham Institute for Medical Research
Scientific Area: Targeted Therapies
\$180,000
Santa Barbara, CA

Pending Execution of Grant Agreements

[A novel epigenetic mark in breast cancer](#)

Mentor: Jessica Tyler, PhD
Fellow: Chandrima Das, PhD
University of Colorado, Health Sciences Center
Scientific Area: Diagnostic and Prognostic Biomarkers
\$180,000
Aurora, CO

[Identification of Small Molecule Transporters Essential to Breast Cancer](#)

Mentor: David Sabatini, PhD
Fellow: Richard Possemato, PhD
Whitehead Institute for Biomedical Research, MIT
Scientific Area: Targeted Therapies
\$180,000
Cambridge, MA

[Understanding Breast Cancer Risk Conferred by the FGFR2 Single Nucleotide Polymorphisms](#)

Mentors: James Iglehart, PhD and Alexander Miron, PhD
Fellow: Yelena Wetherill, PhD
Dana-Farber Cancer Institute
Scientific Area: Epidemiology and Risk Assessment
\$180,000
Boston, MA

[p63-regulated micro-RNAs in human breast cancer](#)

Mentor: Leif Ellisen, PhD
Fellow: Benjamin Ory, PhD
Massachusetts General Hospital
Scientific Area: Pathobiology
\$180,000
Boston, MA

[Optimizing the Penetration of Nanoparticles in Breast Tumors](#)

Mentor: Rekish Jain, PhD
Fellow: Triantafyllos Stylianopoulos, PhD
Massachusetts General Hospital
Scientific Area: Nonsystemic Therapies
\$180,000
Boston, MA

[Influence of Cancer Stem Cell on Non-stem Cancer Cell: Growth, Metastasis and Stemness](#)

Mentor: Robert Weinberg, PhD
Fellow: Hua Jung Li, PhD
Whitehead Institute for Biomedical Research, MIT
Scientific Area: Pathobiology
\$180,000
Cambridge, MA

Pending Execution of Grant Agreements

[Targeting Wnt signaling pathway for breast cancer therapy](#)

Mentor: Gregory Verdine, PhD
Fellow: Johannes Yeh, PhD
Harvard University Cambridge, MA
Scientific Area: Targeted Therapies
\$180,000

[Investigating the mechanism of regulation of HOXA5 and consequences of its loss in breast cancer](#)

Mentor: Josh Lauring, MD, PhD
Fellow: Xiaohui Liang, MD, PhD
Johns Hopkins University, Kimmel Cancer Center Baltimore, MD
Scientific Area: Pathobiology
\$180,000

[Role of p68 RNA helicase in the growth of breast epithelial cells](#)

Mentor: Jason Weber, PhD
Fellow: Anthony Saporita, PhD
Washington University in St. Louis, School of Medicine Saint Louis, MO
Scientific Area: Pathobiology
\$180,000

[Effect of Matrix Compliance and Mechanical Tension on Fibrosis of the Breast](#)

Mentor: Celeste Nelson, PhD
Fellow: Esther Gomez, PhD
Princeton University Princeton, NJ
Scientific Area: Pathobiology
\$180,000

[Functional Status of Autophagy in Tumors as a Determinant in the Treatment of Breast Cancer](#)

Mentor: Vassiliki Karantza-Wadsworth, MD, PhD
Fellow: Ning Chen, PhD
Robert Wood Johnson Medical School New Brunswick, NJ
Scientific Area: Pathobiology
\$180,000

[Role of Alternative Splicing in Mammary Epithelial Cell Transformation](#)

Mentor: Adrian Krainer, PhD
Fellow: Olga Anczukow-Camarda, PhD
Cold Spring Harbor Laboratory Cold Spring Harbor, NY
Scientific Area: Pathobiology
\$180,000

Pending Execution of Grant Agreements

[TGF-alpha: A key molecule in basal breast cancer?](#)

Mentor: Paraic Kenny, PhD
Fellow: Orsolya Giricz, PhD
Albert Einstein College of Medicine at Yeshiva University
Scientific Area: Pathobiology
\$180,000
Bronx, NY

[The Role of MiRNAs in Mediating Resistance to Taxol®](#)

Mentor: Susan Horwitz, PhD
Fellow: Lingling Liu, PhD
Albert Einstein College of Medicine at Yeshiva University
Scientific Area: Diagnostic and Prognostic Biomarkers
\$180,000
Bronx, NY

[Role and mechanism of chemokine RANTES in immunity against breast cancer](#)

Mentor: Xiaojing Ma, MD, PhD
Fellow: Yan Zhang, PhD
Cornell University, Weill Medical College
Scientific Area: Immunotherapies
\$180,000
New York, NY

[Pre-clinical development of tumor specific Hsp70 inhibitors in breast cancer](#)

Mentor: Gabriela Chiosis, PhD
Fellow: Tony Taldone, PhD
Memorial Sloan-Kettering Cancer Center
Scientific Area: Targeted Therapies
\$180,000
New York, NY

[Paxillin regulates matrix metalloproteinase trafficking/recycling and breast cancer cell invasion in three-dimensional microenvironments](#)

Mentor: Christopher Turner, PhD
Fellow: Nicholas Deakin, PhD
State University of New York, Upstate Medical University
Scientific Area: Pathobiology
\$180,000
Syracuse, NY

[Tumor-derived Autophagosomes as a Novel Vaccine in Breast Cancer](#)

Mentor: Hong-Ming Hu, PhD
Fellow: Yuhuan Li, PhD
Providence Portland Medical Center
Scientific Area: Targeted Therapies
\$180,000
Portland, OR

Pending Execution of Grant Agreements

[BRCA1 Ubiquitin E3 Ligase Activity in DNA Damage Repair and Breast Cancer Suppression](#)

Mentor: Roger Greenberg, MD, PhD

Fellow: Yinggun Wang, PhD

University of Pennsylvania School of Medicine

Philadelphia, PA

Scientific Area: Pathobiology

\$180,000

[Role of PIK3CA oncogenic mutations in HER2-mediated transformation and drug resistance in breast cancer](#)

Mentor: Carlos Arteaga, MD

Fellow: Anindita Chakrabarty, PhD

Vanderbilt University Medical Center

Nashville, TN

Scientific Area: Pathobiology

\$180,000

[Prognostic Value of FOXC2 in Breast Tumors](#)

Mentor: Sendurai Mani, PhD

Fellow: Brett Hollier, PhD

M.D. Anderson Cancer Center, University of Texas

Houston, TX

Scientific Area: Diagnostic and Prognostic Biomarkers

\$180,000

[Application of high-content, imaging-based assays to the study of environmental and dietary estrogens in breast cancer](#)

Mentor: Michael Mancini, PhD

Fellow: Felicity Ashcroft, PhD

Baylor College of Medicine

Houston, TX

Scientific Area: Nutritional, Behavioral and Lifestyle Prevention

\$180,000

[PELP1: A novel therapeutic target for breast cancer metastasis](#)

Mentor: Ratna Vadlamudi, PhD

Fellow: Dimple Chakravarty, PhD

University of Texas Health Science Center

San Antonio, TX

Scientific Area: Targeted Therapies

\$120,000

[Defining the Role of Post-Translational Modifications in SRC-3-mediated Repression of mRNA Translation](#)

Mentor: Bert O'Malley, MD, DSc

Fellow: Amber Johnson, PhD

Baylor College of Medicine

Houston, TX

Scientific Area: Pathobiology

\$180,000

Pending Execution of Grant Agreements

[Molecular Imaging and Therapy Targeting Breast Tumor Angiogenesis](#)

Mentor: Weibo Cai, PhD
Fellow: Hao Hong, PhD
University of Wisconsin at Madison
Scientific Area: Nonsystemic Therapies
\$120,000
Madison, WI

PDF—Translational Research

[Targeting TRAIL Receptors in Metastatic Breast Cancer](#)

Mentor: Vincent Cryns, PhD
Fellow: Dmitry Malin, PhD
Northwestern University
Scientific Area: Nonsystemic Therapies
\$180,000
Chicago, IL

[Conjugated Tumor-Targeting Aptamers for Breast Cancer Imaging](#)

Mentors: Paula Bates, PhD and Kyung Kang, PhD
Fellow: Mohammad Malik, PhD
University of Louisville, School of Medicine
Scientific Area: Nonsystemic Therapies
\$180,000
Louisville, KY

[Molecular Characterization of Breast Tumors Subtypes in an Effort to Tailor Specific Cancer Therapies](#)

Mentor: Charles Perou, PhD
Fellow: Stephanie Dance, PhD
University of North Carolina
Scientific Area: Pathobiology
\$120,000
Chapel Hill, NC

[Mechanism of Hsp90 Inhibitor-induced ErbB2 Degradation and anticancer effects in breast cancer](#)

Mentor: Hamid Band, MD, PhD
Fellow: Tameka Bailey, PhD
University of Nebraska at Medical Center, Eppley Cancer Center
Scientific Area: Pathobiology
\$180,000
Omaha, NE

[Stress kinase signaling pathways in the development of hormonal resistance in breast cancer](#)

Mentors: C. Kent Osborne, MD and Rachel Schiff, PhD
Fellow: Luca Malorni, PhD
Baylor College of Medicine
Scientific Area: Endocrine Therapies
\$180,000
Houston, TX

Pending Execution of Grant Agreements

[Development of EGFR Tyrosine Kinase Inhibitor as a Targeted Therapy in Inflammatory Breast Cancer](#)

Mentor: Naoto Ueno, MD, PhD
Fellow: Dongwei Zhang, MD, PhD
M.D. Anderson Cancer Center, University of Texas
Houston, TX
Scientific Area: Targeted Therapies
\$180,000

[Overcoming PTEN-loss Mediated Herceptin Resistance by Targeting Src Family Kinase](#)

Mentor: Dihua Yu, MD, PhD
Fellow: Siyuan Zhang, MD, PhD
M.D. Anderson Cancer Center, University of Texas
Houston, TX
Scientific Area: Targeted Therapies
\$120,000

Post-Baccalaureate Training in Disparities Research

These awards, new in FY09, provide an opportunity for individuals early in their education, particularly those from populations affected by disparities in breast cancer outcomes, to seek careers focused on understanding and eliminating these disparities. *Funding: \$135,000 per student over 3 years.*

[A Post-Baccalaureate Training Program to Address Breast Cancer Disparities in Mexican/Mexican American Women](#)

Maria Elena Martinez, PhD
Co-PI: Patricia Thompson, PhD
University of Arizona at Tucson
Tucson, AZ
Scientific Area: Epidemiology and Risk Assessment
\$404,709

[University of Louisville: Susan G. Komen Breast Cancer Disparities Epidemiology Research Training Program](#)

Kathy Baumgartner, PhD
Co-PI: Richard Baumgartner, PhD
University of Louisville
Louisville, KY
Scientific Area: Epidemiology and Risk Assessment
\$269,937

[Johns Hopkins Bloomberg School of Public Health Training Program in Breast Cancer Disparities Research](#)

Ann Klassen, PhD
Johns Hopkins University, Bloomberg School of Public Health
Baltimore, MD
Scientific Area: Epidemiology and Risk Assessment
\$405,000

Pending Execution of Grant Agreements

[A transdisciplinary training programs for public health researchers and practitioners wanting to impact breast cancer disparities](#)

Luisa Franzini, PhD

Co-PI: Sally Vernon, PhD

University of Texas, Health Science Center

Scientific Area: Epidemiology and Risk Assessment

\$337,426

Houston, TX

Public Abstracts

Co-Investigators: Bryan Schneider, David Flockhart

Mechanism: Promise Grants

Institution(s): Indiana University

Application Title: Comprehensive Biomarker Discovery Project for Bevacizumab in Breast Cancer

Abstract:

Background: Anti-angiogenic therapy with bevacizumab has been shown to double time to progression in advanced breast cancer, as well as significantly increase response rate. These benefits were the basis for a recent accelerated approval of the drug by the FDA. At the same time, bevacizumab is expensive, has novel and significant side effects (high blood pressure, strokes, and other life threatening blood clots), and has not been shown to improve survival in advanced disease. As with most anticancer agents, it is likely that some patients gain significant benefit while others gain little or no benefit while continuing to experience side effects. Current trials are attempting to extend the use of bevacizumab into the adjuvant (curative) setting. In this setting, it will be crucial to identify which patients benefit and which patients experience significant toxicity if anti-angiogenic therapy is to become a standard part of therapy for women with early stage disease. This proposal attempts to meet these objectives through a comprehensive approach to biomarker discovery in the context of the crucial Phase III proof-of-concept trial, E5103. This trial represents a unique, and possibly irreplaceable, opportunity to solve the problem of balancing risk and benefit for anti-angiogenic therapy. This approach brings together a world-class group of translational researchers and advocates to tackle this important problem.

Objectives: We believe that it is imperative to find a successful biomarker to predict who should receive bevacizumab in the adjuvant (curative) setting. A validated biomarker for bevacizumab would allow for the selection of women who would have the greatest likelihood of gaining benefit and thus minimize exposure to toxic side effects in those who are unlikely to gain benefit. In addition, eliminating use in those who would likely not benefit would directly translate into a substantial savings for our health care system thus creating a win-win situation.

Preliminary data: Our group studied the role of genetic variability (biomarkers) in the gene that serves as the target for bevacizumab; vascular endothelial growth factor (VEGF). We evaluated these VEGF variations in the Phase III proof-of-concept trial of bevacizumab in advanced breast cancer (E2100). Although that trial did not find an improvement in overall survival (OS), our biomarkers defined a subgroup of patients that DID experience a substantial improvement in OS and we identified additional biomarkers that appeared to predict a subgroup of patients that did not experience high blood pressure, the most common serious toxicity. These are the first biomarkers discovered for toxicity and efficacy for any anti-angiogenic agent for any human cancer.

Pending Execution of Grant Agreements

Study proposal: We will study our genetic biomarkers in an ongoing adjuvant trial, E5103. E5103 is the primary proof of concept Phase III trial evaluating whether adding bevacizumab to standard chemotherapy will improve disease-free and OS for women with potentially curable disease, and will enroll almost 5000 women. Though the parent clinical trial is already funded, this proposal would allow careful study of tissue samples and clinical information collected in this trial. This proposal will pair our exciting preliminary data with this FDA registration trial for the adjuvant use of bevacizumab. We will also use a cutting edge genetic platform (a Genome Wide Association Study) to uncover the most clinically accurate biomarker(s) possible. Novel drugs such as bevacizumab have chronic and unique side effects, which have been poorly studied with regard to patients' perception of benefit and risk. We have included formal quality of life studies in our proposal and also a prospective assessment of how those perceptions change as biomarker discovery improves the benefit to toxicity ratio. We will also design a formal educational program with our patient advocacy collaborators and will use it to educate both patients and care givers.

Impact: Finding optimal biomarkers for anti-angiogenic therapy has enormous clinical and financial implications. We will match the crucial FDA registration trial for adjuvant use of bevacizumab with the most promising biomarker data generated to date. We will incorporate formal quality of life evaluations to understand the physical and psychological price women are willing to pay for this eventual benefit. We believe this proposal has the potential to identify which women will benefit from adjuvant bevacizumab, and which women will experience toxicity without benefit. The time is ripe to apply cutting edge therapeutic individualization to an important and emerging novel therapy, decreasing morbidity, decreasing cost, and improving quality of life for women with early stage breast cancer.

Co-Investigators: Andres Forero, Tong Zhou

Mechanism: Promise Grants

Institution(s): University of Alabama at Birmingham

Application Title: Targeting Death Receptors for the Treatment of Triple Negative Breast Cancer

Abstract:

The use of modern techniques to evaluate genes in tumor cells has significantly enhanced our understanding of breast cancer; thus, five different breast cancer tumor types have been recognized. One of these types is called basal-like which is typically ER, PR and HER-2-Neu negative or “triple negative”. The majority of triple negative breast cancers (TNBC) are basal-like while non-TNBC breast cancers are almost never basal-like breast cancer. TNBC is a “special interest” of the Susan G. Komen Foundation because it represents a significant proportion of all breast cancer patients (20-25%), the patients have a poor prognosis with high rate of relapse and short survival, and no targeted therapy has been found. Thus, new agents or combination of agents are needed in TNBC.

Dr. Zhou (Co-Principal Investigator of this application) has developed a novel murine antibody (called TRA-8) that is able to recognize the death receptor 5 which it is found on the surface of the tumor cells, including TNBC cells. The antibody in pre-clinical studies was able to induce death of various tumor cells. Interestingly, the ability of TRA-8 to kill tumor cells was augmented when it was combined with chemotherapy agents. In collaboration with our industrial sponsor, Daiichi Sankyo Co., Ltd., TRA-8 has been humanized (called CS-1008) and has moved into development as an anti-cancer drug. Dr. Forero (Principal Investigator of this application) has recently completed the phase I trial of CS-1008 in patients with refractory malignancies and found that it is well tolerated, even at very high doses. The main goal of this proposal is to develop an effective treatment for patients with TNBC by selectively targeting DR5 with CS-1008 in combination with chemotherapy. The main hypothesis is that TNBC cells have DR5 on their cell surface which is uniquely sensitive to anti-DR5 triggering of apoptosis and that DR5 can be targeted with CS-1008 to directly induce death of the tumor cells.

This proposal has 4 four projects: in project #1 we will examine the ability of TRA-8 to kill multiple TNBC cell lines and TNBC implanted in animal models as a single agent and in combination with different chemotherapy agents, with other new targeted agents (called small molecule modulators of cell death) and with another antibody that recognizes a second death receptor called DR4. In project #2, we will study different mechanisms associated with sensitivity of TNBC to CS-1008, including some special proteins (DDX3 complexes and caspase-clAP complexes) discovered by Dr. Zhou. In project #3, we will initiate clinical development of CS-1008 in TNBC patients; we will start with a phase II trial using CS-1008 in combination with chemotherapy in patients with relapsed/refractory metastatic TNBC. The second study will combine chemotherapy with CS-1008 as neoadjuvant therapy in patients with newly diagnosed TNBC before the definitive surgical procedure. In project #4, we will use novel imaging techniques to study the clustering of DR5 on the membranes of TNBC cells (prelude to apoptosis) and utilize MRI as a means to detect

Pending Execution of Grant Agreements

early signs of CS-1008 mediated anti-tumor efficacy in animal models and the two proposed clinical trials (project #3). We believe that these studies will provide adequate data to support the initiation of phase III trials in patients with TNBC in the metastatic and adjuvant settings, potentially provide markers that will help us to identify patients that will benefit from this therapy and imaging techniques for the early detection of anti-tumor efficacy. We believe that this proposal will change the treatment of patients with TNBC, improve their prognosis in the metastatic setting and enhance the curability of newly diagnosed patients with TNBC.

Co-Investigators: Dihua Yu, Victoria Seewaldt

Mechanism: Promise Grants

Institution(s): M.D. Anderson Cancer Center, University of Texas and Duke University

Application Title: Development of novel early detection and prevention strategies for ER negative breast cancer

Abstract:

In order to effectively prevent breast cancer, we need to figure out what goes wrong before breast cancer becomes invasive: Despite advances in treatment, too many women still die of breast cancer. Ultimately, the most effective way to reduce breast cancer mortality is to prevent breast cancer. Although Tamoxifen (Tam) leads to 50% reduction in estrogen-sensitive breast cancer in high-risk women, not all women benefit from Tam. Currently, there is no way to prevent estrogen negative/resistant (ER-) breast cancer. Our goal is to 1) indentify pathways that go wrong before breast cancer becomes invasive, 2) develop biomarkers and new breast imaging techniques to identify the beginnings of ER- breast cancer, and 3) use our new knowledge to rapidly test drugs to prevent the ER- breast cancer. Our dream is that no woman should get breast cancer in the first place. Why we have made little progress in breast cancer prevention: Despite many studies in the laboratory, we have little information on how breast cancer starts in women. Thus, it is almost impossible to develop effective prevention strategies. Furthermore, our current Tam “one-size-fits-all” approach to prevention is not working; many women take Tam without benefit and many drugs are not tested because the only way we have for testing prevention drugs is in large, expensive studies, such as the P1 or STAR trials that require 16,000 women and hundreds of millions of dollars.

We want to do better: Here we aim to develop the scientific framework, biomarkers, targeted imaging, and clinical trials to predict, track, and prevent development of ER- breast cancer. We will develop biomarkers to identify in an individual woman which signaling pathways are dysregulated (out of balance), select a prevention drug that targets the dysregulated pathway, and then test whether the prevention drug is working. In the four Aims of this proposal we will 1) Identify protein signaling networks that become dysregulated during ER- breast cancer; 2) Investigate the biology of why some atypia progresses to ER- breast cancer and test new imaging strategies to identify atypia that is likely to progress, 3) screen potential prevention drugs in the laboratory, and 4) then test our most promising prevention drugs in women who are at high risk of developing ER- breast cancer. If these trials are successful we will open these drugs to all women who are at high risk and cannot take or do not want to take Tam.

Developing tools is key to rapid testing: We currently use the research technique, Random Periareolar Fine Needle Aspiration (RPFNA) to remove live mammary cells from the breasts of high-risk women. RPFNA takes about 10 minutes to perform in a doctor’s office, and the breast tissue is “numbed” prior to performing RPFNA. Importantly, women who undergo RPFNA report minimal discomfort (< or =1 on a pain scale of 10 for RPFNA versus 4-5 for core needle biopsy) and over 95% of women who had RPFNA are willing to undergo subsequent RPFNA.

RPFNA allows us to rapidly test whether a prevention drug is working in an individual. Our current trial design requires that ~10,000 women take a prevention drug for 5 years, and at

Pending Execution of Grant Agreements

the end of 5 years the number of women who get cancer are counted up. As a result we can only test one drug at one dose every 5 years; the trials are not designed to figure out whether a drug worked in an individual woman and if a drug failed there is no way to figure out why. With RPFNA, we can rapidly test prevention drugs with trials that require only 100-200 women, last 6 months, and we do not have to wait until women get cancer.

Importantly, since we directly test cells for pre-cancerous changes before and after the drug, we can test the cells for dysregulation of signaling pathways, and 1) match the pathways that are dysregulated in an individual woman with the drug that would work the best for her, and 2) if the drug fails, we can figure out why.

The power of a team: Our team consists of basic scientists, clinicians who specialize in early detection and prevention, imaging bioengineers, clinicians who develop and test new targeted drugs, and importantly, breast cancer advocates. The strength of our team insures that 1) information obtained from the clinic will immediately be brought to the lab to further identify ER-signature alterations during breast cancer initiation and 2) studies in the lab will immediately be used to benefit women at high-risk for breast cancer. We will use our in vitro and animal models to test for new therapeutic targets and develop targeted imaging to prevent and detect ER- breast cancer at its earliest stage.

Most importantly, our Advocate Team has worked with us for the past 4 years to develop much of the preliminary data and initial trials that form the basis of this grant application. Our Advocate Team consists of women with significant scientific training who also have a personal interest in early detection and breast cancer prevention. The PI (Yu) and a Co-PI (Seewaldt) also have mothers who have had breast cancer, and they themselves are at increased risk. Dr. Seewaldt has precancerous changes in her breast and cannot take Tam. Thus, the proposal is close to the hearts of the PIs of this study and for our daughters' generation. Prevention represents a new frontier and has new ethical and quality of life issues. It is through discussion with Advocates and consideration of issues both as advocates and scientists that we will make a change and not leave our daughters' generation with the same old "one-size-fits-all" prevention approach that fails so many of us today.

Co-Investigators: Hallgeir Rui, Edith Mitchell

Mechanism: Promise Grants

Institution(s): Thomas Jefferson University

Application Title: Therapy-relevant Stratification of Breast Cancer Patients: Integrating Pathology and Biomarker Analyses

Abstract:

A majority of breast cancer treatments will benefit only a portion of the patients to whom they are given. There is a great need to optimize the selection of breast cancer patients who will respond to existing treatments and treatments that are under development. Effective personalized breast cancer treatment is needed since more than 40,000 patients are dying from breast cancer each year in the US alone. Improved classification methods for breast cancer based on drug targets within patients' tumors will help match the right patients with the right drugs. The concept of improved profiling of patients' tumors holds both for improving success rates of therapies with existing drugs, and for successful testing of new drugs in clinical trials.

The objective of this project is to enhance therapy-relevant classification of human breast cancer based on analyses of levels of drug target proteins in a large number of breast cancer cases. Improved classification of breast cancer based on levels and activation status of drug-related target proteins within cancer cells will facilitate better prediction of which patients are most likely to benefit from existing or new drugs, alone or in combination. There are two Aims:

Aim 1 - Breast Cancer Classification Effort: 1a) We will use our unique and innovative high throughput method for parallel analyses of levels of 250 therapy-relevant protein markers in 5,000 cases of breast cancer. State-of-the art robotic technology for quantifying levels of proteins within tumors will be used. Advanced biostatistical and bioinformatics methods will be used to classify breast cancer into groups that are predicted to be relevant for existing and new therapies. 1b) We will investigate levels of the 250 therapy-relevant protein markers in primary breast cancer and metastatic breast cancer from 100 patients. This will allow us to weed out therapy-relevant markers expressed in the primary breast tumors but that are lost in the metastatic cancer. Therapeutic target proteins that no longer are active or present in metastatic cancer are not expected to be effective drug targets for treating metastatic breast cancer. The outcomes of Aim 1b will help the classification effort of Aim 1, giving robust markers greater weight.

Aim 2 - Clinical Trial And Drug Response Prediction: Aggressive forms of breast cancer that have failed prior therapy will be examined in a clinical trial with a new drug, AMG 706. So-called triple-negative breast cancer, which is particularly frequent in African-American women, as well as other forms of aggressive breast cancer, will be analyzed for responsiveness to AMG 706 in a clinical trial of 74 patients.

The overall project will lead to broad, therapy-relevant classification of breast cancer that will improve personalized cancer therapy for individual patients. First, the project will help predict responsiveness to existing therapies. Second, and more importantly, the classification will guide selection of patients into novel clinical trials that will rapidly determine efficacy of new drugs because of higher probability of responses. Because the marker profile is tailored to molecular targets of existing drugs, new drugs may not need to

Pending Execution of Grant Agreements

be developed from scratch before therapeutic progress is made. This project is expected to rapidly impact breast cancer patient care within the next decade.

Pending Execution of Grant Agreements

PI Name: Michael Lisanti

Mechanism: Investigator Initiated Research

Institution: Thomas Jefferson University

Application Title: Role of CAV1 in Suppressing Estrogen-Hypersensitivity, DCIS, and Breast Cancer Progression

Abstract:

Due to the use of mammography screening, DCIS now represents between ~20-30% of new breast cancer cases. Ductal Carcinoma In Situ (DCIS), also known as mammary intraepithelial neoplasia (MIN), is considered a pre-malignant lesion that may or may not progress to full-blown breast cancer. Factors that govern the progression of DCIS lesions remain unknown, but involve the onset of an invasive growth phenotype. Because of this uncertainty, patients with a diagnosis of DCIS now undergo breast-conserving surgery, radiation therapy, and adjuvant therapy with tamoxifen, an anti-estrogen. Thus, new diagnostic markers are needed to determine which DCIS lesions are prone to progression. For this purpose, we will study the association of six new biomarkers with pre-malignant lesions and progression from pre-malignancy to full-blown breast cancer. We expect that these new molecular markers will allow us to improve diagnostic accuracy for individual patients, enhancing both the prognostic predictions as well as the prediction of drug responsiveness for a given patient.

Human Subjects: The human breast tissue samples are obtained from the Surgical Pathology Laboratory of Thomas Jefferson Hospital. This breast tissue material is de-identified and represents excess biopsy/surgical material that was discarded after samples were taken for diagnostic purposes. Approximately 300 new breast cancer cases are seen every year at our Institution and the majority of these patients have a biopsy or excision performed at our Institution. Each sample is well characterized pathologically and there is hormone receptor (ER and PR) and Her-2/Neu information for each sample. Clinical and therapeutic information is also available for each patient.

Sources of data: All the information corresponding to the patients whose tissues are available is retrieved from the Jefferson Cancer Registry and the Pennsylvania Cancer Alliance (PCA). The PCA is a funded study of several institutions within the state of PA that has allowed us to implement a comprehensive database available to researchers statewide. In the recent months, a transition has been made to a NCI funded initiative (Cancer tissue Core, Breast Cancer Research Group) with basically three aims: common comprehensive databases; storage and retrieval of breast cancer tissues (non-breast cancers as well) and the third arm is the extraction of relevant clinical information (data mining) for each case. There is approval of the internal IRB of all the proposed aims to comply with federal regulations.

Protection of Human Subjects: All the information remains de-identified for privacy issues; therefore the risk of loss of confidentiality is negligible.

PI Name: Gerard Blobe

Mechanism: Investigator Initiated Research

Institution: Duke University, School of Medicine

Application Title: TbetaRIII/sTbetaRIII in the Diagnosis and Treatment of Breast Cancer

Abstract:

The transforming growth factor-beta (TGF-beta) superfamily is composed of a number of proteins, including TGF-beta and bone morphogenetic proteins (BMPs) that function by binding cell surface proteins (their receptors) and regulating the growth and survival of cells. TGF-beta superfamily signaling pathways have an important role in inhibiting breast cancer formation. However, cancers often alter their expression or develop mutations in components of these pathways, resulting in resistance to the growth inhibitory effects of TGF-beta superfamily members. TGF-beta superfamily members then promote the spread of breast cancers that have been established. This dual role for TGF-beta superfamily members remains a fundamental obstacle to targeting these pathways for the treatment of human breast cancers. We recently reported that most human breast cancer cells lose expression of one of the proteins on the cell surface that binds TGF-beta superfamily members, the TGF-beta co-receptor, TbetaRIII, with the gene for TbetaRIII being deleted in about half of breast cancer patients and about 90% of breast cancer patients having decreased or lost TbetaRIII expression. Importantly, low TbetaRIII levels predicted a poorer prognosis for breast cancer patients and restoring TbetaRIII expression dramatically inhibited tumor invasiveness and metastasis in breast cancer models in vivo. TbetaRIII appeared to function to inhibit cancer progression, at least in part, by being cleaved by a protease at the cell surface, releasing the soluble extracellular domain of TbetaRIII, sTbetaRIII. sTbetaRIII can then bind TGF-beta superfamily ligands and inhibit their function. As sTbetaRIII is a soluble protein, we could potentially use sTbetaRIII as a therapeutic agent. We have also demonstrated that sTbetaRIII can be detected in human blood specimens and have devised an assay to examine expression of sTbetaRIII in human blood specimens. Here we propose to investigate the relative contribution of cell surface TbetaRIII and sTbetaRIII to the formation and spread of breast cancers in both mouse models of breast cancer and in human breast cancer patients. We have created mouse models that have either increased expression of TbetaRIII, and have the ability to administer sTbetaRIII. We will cross these mice with mouse models of breast cancer and measure the effects on the formation of mammary tumors and spread of these tumors from the primary site. We will also examine the primary tumors to assess the effects on growth, survival, invasion and blood vessel formation. We will then investigate where TGF-beta superfamily members bind to TbetaRIII and sTbetaRIII by creating TbetaRIII and sTbetaRIII mutants and assessing their ability to bind ligand. We will then use excess amounts of individual ligands to compete of binding of other ligands to define the hierarchy of ligand binding. Using this information, we will assess the relative contribution of cell surface TbetaRIII and sTbetaRIII in binding the different TGF-beta superfamily members on TGF-beta superfamily signaling and breast cancer biology. Finally, we propose to investigate whether sTbetaRIII or TGF-beta superfamily ligand levels could be used as biomarkers to diagnose breast cancer or to evaluate how well a patient will do before or with treatment. Taken together, these studies will allow us to target the TGF-beta superfamily signaling pathways in a novel manner, allowing us to plan a pre-clinical

Pending Execution of Grant Agreements

strategy for using TbetaRIII or sTbetaRIII as a therapeutic agent, either alone, or in combination with a chemotherapy, targeted therapy or an immunotherapy approach. As sTbetaRIII is normally produced it could be delivered as a recombinant protein or as gene therapy to bind active TGF-beta superfamily ligands in the tumor environment. These studies could also establish sTbetaRIII as a screening tool, diagnostic or prognostic/predictive marker in human breast cancers.

PI Name: Robert Clarke

Mechanism: Investigator Initiated Research

Institution: Georgetown University

Application Title: Cell Death Signaling in Endocrine Responsiveness

Abstract:

PUBLIC ABSTRACT Rationale and Study Hypothesis. Of the 178,000 women who will be newly diagnosed with invasive breast cancer in 2008, ~70% of them will have breast tumors that are estrogen receptor alpha-positive (ER+). An ER+ tumor is an indicator of good prognosis and women with these types of tumors are excellent candidates for endocrine therapy with either an antiestrogen (e.g., Tamoxifen) or an aromatase inhibitor (e.g., Letrozole). Unfortunately, up to half of all ER+ breast cancers will either not respond to endocrine therapy or, after successful treatment, will recur as endocrine resistant breast cancer (i.e., exhibit an ER- phenotype in the context of their endocrine responsiveness). Our goal is to find better predictors of endocrine responsiveness in women and to understand better the cause of endocrine resistance by studying how breast cancer cells regulate the balance between cell survival and cell death in response to these therapies. Our preliminary data suggest that expression of the interferon regulatory factor 1 (IRF1) gene plays an important role in this process by causing breast cancer cells to die. Recently, we have found that IRF1 may send this death signal through members of the BCL2 gene family, which are known to regulate two different types of cell death: apoptosis and autophagy. Finally, when we look at the expression of IRF1 in breast tumors, we find that one of the two copies of this gene is missing in 30% of cases. This leads us to hypothesize that loss of IRF1 in breast tumors is associated with breast cancer progression, poor prognosis, and a suboptimal response to endocrine therapy. Thus, we also hypothesize that these clinical outcomes reflect a role for IRF1 as an important regulator of breast cancer cell survival that acts through the BCL2 gene family in response to endocrine therapy. We will test our hypotheses in two Specific Aims. In Aim 1 (clinical studies), we will examine IRF1 expression and gene loss in breast cancer specimens and its association with well-established markers of prognosis and clinical outcome. Aim 2 (mechanistic studies) is designed to establish the molecular events that determine how IRF1 regulates the balance of cell death and survival through BCL2 family genes in response to endocrine treatments, and which death pathway (apoptosis or autophagy) is most important.

Advancing the Understanding of Breast Cancer and Reducing Mortality. An incomplete understanding of how breast cancer cells adapt to therapy and “learn” endocrine resistance contributes directly to our inability to effectively direct endocrine therapy and to eradicate all ER+ breast tumors. We and others have shown that on a molecular level, endocrine resistant breast cancer cells change the balance between cell survival and cell death, and we strongly believe that a key gap in our knowledge is an understanding of what causes this imbalance. Our focus on IRF1 as a key regulator of cell death in response to endocrine therapy, acting through BCL2 family members, is an innovative new hypothesis that has not previously been studied. Our novel studies will help to reduce breast cancer mortality by providing new insights into cell signaling as it affects endocrine responsiveness, and by identifying new targets for drug discovery. The development of new drugs could delay or reverse endocrine resistance, thereby improving overall survival and reducing mortality in

Pending Execution of Grant Agreements

breast cancer patients. While drug development is a valuable long-term goal, our clinical studies are designed to make a more dramatic and immediate impact on breast cancer mortality within the next decade by improving our ability to predict endocrine resistance in breast cancer patients before it emerges. For example, our studies should show that low expression of IRF1 and/or loss of one or both copies of the IRF1 gene is a powerful predictive marker that can identify patients in whom endocrine therapy will be unsuccessful. Thus, testing newly diagnosed ER+ breast cancer patients for IRF1 could allow women and their doctors to make better treatment decisions and select a therapy regimen that will significantly improve their long-term survival.

Importance of the Research to Breast Cancer Patients: While there are significant survival benefits to be gained from both endocrine therapy and chemotherapy, not everyone receives this benefit. Unfortunately advanced breast cancer remains a largely incurable disease, and the current treatment regimens and schedules have led only to small or incremental decreases in breast cancer-related mortality. Our studies of IRF1, its reduced expression in breast tumors, and how IRF1 affects cancer cell death through the BCL2 gene family, will introduce new ideas about how better to direct endocrine therapy for women and about the regulation of breast cancer cell survival. These research outcomes have important implications for how we understand the regulation and integration of cell signaling, and they will ultimately lead to improvements in clinical practice.

PI Name: Patricia Elizalde

Mechanism: Investigator Initiated Research

Institution: Institute of Biology and Experimental Medicine

Application Title: Crosstalks among Stat3, PR and ErbB-2: a novel biomarker and tool for a new therapy against breast cancer in Latin American women

Abstract:

Accumulating evidence indicates that bidirectional interactions between steroid hormone receptors estrogen (ER) and progesterone (PR), and the ErbB family of receptor tyrosine kinases play a key role in breast cancer. However, the molecular mechanisms underlying these interactions as well as the existence of common downstream targets remain poorly understood. Notably, recent findings have underscored the necessity to disclose PR action and interaction with ErbBs in breast tumors. Extensive clinical studies evidenced that postmenopausal women undergoing a combined estrogen and progestin hormone replacement therapy suffer higher incidence of breast cancer than women who take estrogen alone. Breast cancer is a devastating disease with major impact on global health. Unsurprisingly, matters concerning this disease are strikingly different in industrialized and in developing countries. In the United States for instance, improvement in prevention, diagnosis, and treatment has significantly decreased breast cancer incidence and mortality, while in Latin American (LA) countries mortality rates have been increasing over the last 40 years. Socioeconomic factors underlie this discrepancy. Poor prevention and screening in LA result in diagnosis at later stages of the disease where breast tumors are no longer responsive to anti-hormonal or anti-tyrosine kinases therapies. In industrialized countries, on the other hand, at the time of diagnosis at best only two-thirds of tumors expressing ER and PR are responsive to endocrine therapy. As they progress, these tumors often acquire steroid hormone resistance. Anti-tyrosine kinase therapies such as blockage of ErbB-2 with the monoclonal antibody Trastuzumab (Herceptin) have proved successful, but resistance to this therapy is quite high. These changes in tumor biology due to disease progression in industrialized countries and late diagnosis in LA confront us to a significant number of patients urgently requiring new biomarkers for the prediction of response to therapy and the unraveling of new molecular targets. Our previous work showed that Heregulin, a ligand of ErbBs, activates PR, while on the other hand progestins activate Heregulin/ErbBs signaling in breast cancer cells. We also found that progestins induce signal transducer and activator of transcription 3 (Stat3) transcriptional activation in mammary tumors, which is in turn a key player in progestin-stimulated growth. In addition, our preliminary findings directly related to the present proposal demonstrated that HRG induces Stat3 activation in breast cancer which, notably, is inhibited by abrogation of PR activity, evidencing a ternary interaction among HRG/ErbB-2, PR and Stat3. Based on these data, our hypothesis is that Stat3 constitutes a convergence point between PR and HRG/ErbB-2 signaling in breast cancer. Interaction would occur both at cytoplasmic level, where PR plays a key role in the mechanism of HRG induction of Stat3 activation, and at nuclear level, where Stat3 acts as a coactivator (positive regulator) in HRG-induced PR activation. Our aims are #1 to explore the mechanism leading to Stat3 activation by HRG in breast cancer; #2 to specifically evaluate whether Stat3 acts as a coactivator in HRG-induced transcriptional activation of PR; #3 to investigate the effect of targeting Stat3 in HRG-induced in vivo growth of breast cancer by

Pending Execution of Grant Agreements

using our unique mouse model of mammary tumors induced by progestins in which HRG drives proliferation through its capacity to activate both ErbB-2 and PR. We will stably knockdown Stat3 by RNA interference strategies. In addition, key molecules involved in Stat3 activation unraveled in the studies of aim#1 will be used as targets to design novel drugs and #4 to explore the nuclear co-localization of PR and Stat3 in human breast tumor samples in order to correlate results with survival outcome, clinical, pathological, and molecular parameters, as well as with response to therapy. Our local access to a large source of primary breast cancer samples from LA women diagnosed at later stages in disease progression provides a unique opportunity to develop this new biomarker. Our studies will have major clinical impact in both industrialized and developing countries. Demonstration that Stat3 constitutes a convergence point between steroid hormones and ErbB-2 signaling in breast tumors will highlight Stat3 as a new alternative therapy for a more effective treatment of breast cancer resistant to first line anti-hormonal and second-line anti-ErbB-2 therapies. Furthermore, our work dissecting the molecular mechanisms of Stat3 activation will lead to the development of novel drugs which could be used to overcome resistance to the first (Herceptin) and second (the small molecule inhibitor Lapatinib) generation of anti-ErbB-2 therapies available at present. Demonstration that PR and Stat3 nuclear co-localization correlates with lack of response to endocrine and anti-tyrosine kinase therapies will provide a new predictive biomarker that will help select a much more targeted therapy at the time of diagnosis, thereby making a significant difference in breast cancer treatment. These results would greatly impact LA socioeconomically, since its scarce health resources would be placed on much more targeted therapies based on tumor responsiveness, beneficial also due to their lower toxicity for LA women with co-morbid conditions associated to poverty.

PI Name: Susan Sturgeon

Mechanism: Investigator Initiated Research

Institution: University of Massachusetts

Application Title: Predicting Breast Cancer by Detection of Promoter Hypermethylation in Serum DNA

Abstract:

The purpose of this study is to develop a diagnostic blood test for breast cancer. The concept is based on the premise that breast cancer tumors have certain DNA changes known as promoter hypermethylation, and that breast cancer tumors shed sufficient quantities of DNA into the blood to allow detection of the presence of such epigenetic changes. Epigenetic literally means “on the gene”, and promoter hypermethylation is when a methyl molecule is added to the DNA backbone of a gene causing a loss of normal function. A series of small clinical studies have shown the feasibility of this approach, with moderately high accuracy of breast cancer detection achieved using a relatively small number of genes (usually three to four) in blood. It is likely that expansion of the panel to include other breast-cancer related genes would markedly increase the accuracy of the test. Thus, DNA in serum will be evaluated for promoter hypermethylation in 12 candidate genes from approximately 250 node-positive postmenopausal breast cancer cases, 75 node-negative postmenopausal breast cancer cases, and a comparison group of 250 postmenopausal benign breast disease control subjects who were part of the Mayo Serum Bank, a resource established in the 1970’s to identify early markers of breast cancer. The objective will be to determine whether this panel of genes can be used to accurately detect breast cancer.

While early detection by screening mammography has led to a decline in breast cancer mortality over the past decade, mammography has several well-known limitations, including a high rate of false positives, reduced sensitivity in dense breasts, and concerns over radiation exposure, particularly in high-risk women who may benefit by more than annual screening. Limitations of mammography screening combined with a rapid revolution in available molecular tools have led to renewed and vigorous research interest in developing a complementary molecular biomarker for early detection of breast cancer. Changes in DNA methylation patterns are a common feature of malignant cells, and promoter hypermethylation in key genes is considered one of the most promising biomarkers for a reliable and sensitive screen for early breast cancer.

Detection of methylation status in serum could lead to the development of an inexpensive, minimally invasive blood test to complement mammography screening. A methylation-based blood test would be valuable as it could be used between annual mammograms in high-risk women or to evaluate suspicious mammogram findings. It also could assist in identifying women at high-risk of developing breast cancer who may benefit from additional screening modalities (e.g., MRI) or other prevention strategies, and finally it could be a valuable intermediate endpoint in chemoprevention trials.

Pending Execution of Grant Agreements

PI Name: Pamela Vacek

Mechanism: Investigator Initiated Research

Institution: University of Vermont

Application Title: Improving Breast Cancer Outcomes in Older Women through More Effective Use of Screening Mammography

Abstract:

Breast cancer occurs most often in older women, but they are less likely to undergo screening mammography. As a result, older women tend to be diagnosed with breast cancer at a later stage and are more likely to die of the disease. There are several reasons why older women are screened less often. Few women over the age of 69 were included in studies of the effectiveness of screening mammography, so the benefits and harms of screening for older women are not known. Because of this, there are no specific screening guidelines for women aged 70 and older. Instead, physicians are advised to decide whether an older patient would benefit from screening mammography based on her age, general health, and likelihood of developing breast cancer. For example, a healthy 77 year old woman who has several breast cancer risk factors would be more likely to benefit from screening than a 89 year old women who has several serious health problems and few breast cancer risk factors. For many older women, however, the potential benefits and harms of screening mammography are much more difficult to assess. The goal of this study is to assist health care providers to make appropriate screening recommendation for their older patients.

This will be accomplished by obtaining detailed information about the benefits and harm of screening mammography for women aged 70 and older, so that specific screening guidelines can be developed for this age group. The study is based on the general hypothesis that the usefulness of screening mammography for older women depends on their age, health status, and breast cancer risk. In particular, we hypothesize that: 1.) information about age, health problems, and breast cancer risk factors can be used to identify groups of women who differ in terms of life expectancy and the likelihood of developing breast cancer; 2.) some of the advantages and disadvantages of screening differ among these groups; 3.) screening mammography leads to earlier breast cancer detection in all groups; and 4.) there are differences in cancer treatment among groups that influence the impact of early detection. A large, population-based data set with detailed information about health problems, risk factors, mammography use, diagnostic procedures, and breast cancer stage and treatment necessary is needed to determine the effects of screening mammography in older women. In addition, it must include information that has been collected on the same women over time, so that the outcomes of screening and treatment can be examined. Such a data set does not exist, but it can be constructed by linking mammography registry data with Medicare health claims data. Mammography registries collect the detailed risk factor, mammography and pathology information needed to determine breast cancer risk, screening intervals and breast cancer stage, while Medicare health claims data contain the information needed to assess a woman's health status and identify the breast cancer treatments she receives. For this study we will link Medicare health claims data to data from the Vermont Breast Cancer Surveillance System (VBCSS) to construct a data set containing information on 20,746 women, who were aged 70 or older

Pending Execution of Grant Agreements

and had at least one screening or diagnostic mammogram recorded in the VBCSS during 1996-2001. We will use the Medicare data to assess each woman's health status and the VBCSS to assess her breast cancer risk. We will then develop a system for classifying the women on these two attributes, as well as age. The usefulness of the classification will be evaluated by comparing the groups with respect to life expectancy and breast cancer rates. Medicare vital status data will be used to determine life expectancy and VBCSS breast pathology data will be used to identify which women develop breast cancer. The study hypotheses will then be tested by comparing the benefits and harms of screening in each group of women. The benefits to be examined include detection of earlier stage breast cancer. The harms include the use of biopsy and other diagnostic tests in women who do not have breast cancer and the detection of non-invasive breast cancer. Diagnosis of non-invasive breast cancer, with its associated testing and treatment, is generally viewed as an adverse effect in the elderly because it only progresses to invasive disease in 20-30% of cases over 5-10 years. If the results support the study hypotheses they will be used to design a further study to verify the results of this study and find out whether older women who receive screening mammography live longer than those who do not. That study will use data from the National Cancer Institutes Breast Cancer Surveillance Consortium (BCSC), which currently includes information on more than 300,000 geographically and ethnically diverse women aged 70 and older from five participating mammography registries. The BCSC database is therefore large enough to determine whether screening reduces mortality for specific groups of women based on the age, health status, and breast cancer risk classification system developed in this study. The information from both this study and the subsequent one will enable the American Cancer Society and other organizations to develop specific screening guidelines for older women who differ in terms of the benefits and harms of screening mammography because of life expectancy and breast cancer risk. The guidelines will promote screening among the older women most likely to benefit and thereby reduce breast cancer morbidity and mortality in the 62,000 women aged 70 and older who are diagnosed with the disease each year in the U.S.

PI Name: Eli Gilboa

Mechanism: Investigator Initiated Research

Institution: University of Miami, School of Medicine

Application Title: Development of Agonistic 4-1BB Aptamers to Enhance Vaccine-Induced Tumor Immunity

Abstract:

PUBLIC ABSTRACT Immune-based therapy is emerging as a promising modality to control tumor progression in cancer, including breast cancer. Immune modulatory drugs, mostly in the form of monoclonal antibodies, have taken centerpiece as a new generation of promising drugs to potential anti-cancer immunity. Multiple phase II clinical trial in various cancers including breast cancer, as well as a pivotal phase III clinical trial in melanoma patients, is currently underway using antibodies targeted to the co-inhibitory receptor CTLA-4. Phase I/II clinical trials have been recently launched targeting PD-1, another co-inhibitory receptor, and 4-1BB, a major co-stimulatory receptor which is also the focus of this grant application, using monoclonal antibodies. A major consideration in clinical application of promising treatments is the feasibility of the approach, dictated by cost and complexity of the manufacturing and/or treatment protocol as well as the challenges associated with the regulatory approval process. Use of antibodies in cancer therapy is a case in point. A major limitation of using antibodies in therapeutic settings stems from the fact that antibodies are cell-based products requiring a complex and costly manufacturing and regulatory approval process. Hence such reagents are almost exclusively developed by companies and provided to academic investigators on a selective basis and under strict contractual agreement and company oversight, severely limiting their availability for clinical exploration. Thus, despite promising observations from murine preclinical tumor models, the use of antibodies in clinical settings is limited. Importantly, studies in mice have shown that combined treatment with several immune modulating antibodies can have dramatic antitumor responses, even in the absence of vaccination! (see for example the study of Uno et al. in *Nature Medicine*, 2006, 12:693). Yet the complexity of generating and access to multiple clinical-grade antibodies, often manufactured by different companies, essentially precludes their combined exploration in clinical trials. Aptamers – the centerpiece of this grant proposal - correspond to a new class of therapeutic agents that exhibit the main properties of antibodies, specificity and avidity to their targets, but offer important advantages over antibodies to manipulate the immune system for therapeutic purposes. Foremost, since aptamers are made of short oligonucleotides they can be chemically synthesized, and hence the manufacturing and regulatory approval processes should be much simpler and less costly. In effect a reasonably well funded academic laboratory should be able to generate two or three aptamers for clinical testing! Thus aptamer technology could potentially replace antibodies as reagents to modulate tumor immunity. This grant application, focusing on the application of aptamers directed to a central immune modulatory target in the setting of breast cancer, is a step in that direction. In preliminary studies we have described the development of an aptamer which binds to and activates this immune modulatory target (called 4-1BB) in activated T cells and when injected intratumorally into subcutaneously implanted tumors induced tumor rejection. Notably, on a molar basis the aptamer was at least as potent (in fact marginally superior by every test) as the corresponding antibody. This

Pending Execution of Grant Agreements

grant application represents an essential transition from this proof-of-concept study in murine systems to clinical trials. It will a) develop more potent aptamers, which has important practical implications because it will reduce the amount of aptamer needed for treatment and hence its cost-effectiveness, as well as reduce the potential toxicities, and b) will test the biological effects of aptamer on tumor immunity using highly stringent and relevant tumor models for breast cancer, in particular mice which develop breast cancer spontaneously in a fashion which resembles very closely breast cancer development in humans. Accomplishing the goals set forth in this grant application will provide the guidelines for generating human aptamers and set the stage for clinical trials to test the therapeutic benefit of such aptamers in conjunction with vaccination and/or other treatments, to enhance tumor immunity in patients with breast cancer. To the best of our knowledge, the proposed studies represent a first application to support the development of aptamers capable of potentiating the action of immune modulatory receptors for therapeutic purposes. The protocol to isolate such aptamers is a platform technology. Other potential targets for immune modulation with immune-potentiating aptamers are costimulatory receptors expressed on immune cells such as OX40, CD40, CD27, GITR or cytokine receptors such as IL-12R, IL-15R, IL-7R. The studies proposed in this grant application will, therefore, provide a foundation for the application of this novel and promising technology to improve the immunological control of human disease, focusing but not limited to breast cancer.

PI Name: Douglas Conklin

Mechanism: Investigator Initiated Research

Institution: State University of New York at Albany

Application Title: Analysis of NR1D1, a circadian rhythm metabolic regulator required for breast cancer cell survival

Abstract:

Public Abstract: One of the most clinically relevant features of breast cancer is the amount of the HER2/neu protein found on the surface of breast cancer cells. When a woman is diagnosed with breast cancer, tissue biopsies are examined for the amount of this protein that is present. Increased levels are observed in 25–30% of all breast cancers. For patients whose breast cancers have elevated levels of HER2/neu, median survival times are decreased to three years compared to the nearly 7 years for those with cancers that do not have elevated levels of this protein.

The association of high HER2/neu levels on the cancer cells with aggressive disease and poor clinical outcome has made HER2/neu itself an excellent therapeutic target. Herceptin (trastuzumab) was widely hailed as the first “next generation” cancer therapy when introduced for the treatment of estrogen receptor negative breast cancer. Although it is one of our best weapons at present, its success has been modest. Clinical trials of Herceptin as a single agent therapy have provided overall response rates ranging from 11 to 26% for patients with metastatic HER2/neu -positive breast cancer. Since a relatively large proportion of patients do not benefit from Herceptin, it is likely that other factors must influence therapy response in HER2/neu -positive tumors.

In addition to HER2/neu approximately 150 other genes are known to be consistently overexpressed in these tumor cells. Using a high throughput genomic approach we have tested each of these genes and found that several genes related to the fat metabolism of the HER2/neu-positive breast cancer cells are important for their survival. This is important for two reasons. First our preliminary experiments have shown that we can kill HER2/neu -positive breast cancer cells without affecting normal human breast cells suggesting that this is an excellent drug target for breast cancer. Second since this gene is involved in fat metabolism and circadian rhythm it suggests that its activity may be important to the effect that obesity and the “night shift” have on the incidence of breast cancer.

We have found that inhibiting genes that allow fat storage process is specifically toxic to breast cancer cells that have high levels of the HER2/neu protein. Normal breast cells and other types of breast cancer cells are not affected by inhibiting the fat storage process. Once we understood the genetic basis of this effect we went in search of chemical inhibitors that might have the same effect on this process. One such compound called GW9662, was developed as an antiobesity drug that blocked fat production and storage in fat cells. Importantly, we find that this compound also specifically kills HER2/neu-positive breast cancer while having little effect on normal cells. The goal of our project is to determine whether this drug can be used as a therapy for HER2/neu -positive breast cancer. The work proposed will characterize the NR1D1 pathway as a potential chemotherapeutic target. We will test its ability to kill breast cancer cells alone and in combination with Herceptin treatment. If inhibition of this pathway is found to specifically kill HER2/neu-positive breast cancer cells, it could potentially be moved to the clinic in short order.

Pending Execution of Grant Agreements

We also propose to characterize the impact that overexpression of this gene has on the development of breast cancer and its potential interaction with changes to diet and circadian rhythm. Many studies have indicated that obesity and night shift work are risk factors for the development of breast cancer. Since there are conflicting reports in the literature and since this is likely to be complex, we propose to characterize the impact that this gene has on the development of breast cancer in mice. Mice that have high levels of this gene in breast tissue will be examined for the onset of breast cancers under normal conditions and other conditions in which the mice are fed a calorie-rich diet or have been housed under conditions with altered day and night light cycles.

PI Name: Sean McAllister

Mechanism: Investigator Initiated Research

Institution: California Pacific Medical Center

Application Title: Inhibition of Breast Cancer Cell Aggressiveness by Cannabidiol

Abstract:

Public Abstract Rationale: We discovered that cannabidiol (CBD), a non-psychotropic compound from the plant *Cannabis sativa*, can inhibit the processes that allow breast cancer cells to grow and spread (metastasis). The mechanism that would explain the inhibitory action of CBD in vivo (in a living organism) on breast cancer metastasis has not been elucidated. CBD is a novel inhibitor of a gene whose activity is intimately linked to the aggressiveness of human breast cancers; this gene has been termed *Id-1*. Using cultures of breast cancer cells, we discovered that *Id-1* was a key gene whose expression needed to be reduced in order for CBD to inhibit the spread of breast cancer. In this proposal, we will determine whether CBD inhibits *Id-1* and corresponding breast cancer metastasis in a mouse model. This is a key piece of data that is needed in order to move toward the development of a clinical trial. Additionally, we have discovered analogs of CBD that are potentially more active than CBD at inhibiting *Id-1* and corresponding breast cancer cell aggressiveness. An anticancer agent, with a low toxicity profile such as CBD that can both inhibit cancer cell growth and metastasis, would be extremely valuable clinically. Understanding the mechanisms behind the anticancer activity of CBD may also lead to the discovery of new biological targets for the development of diagnostic tools and additional therapies for the treatment of breast cancer. Hypotheses: Our hypotheses are as follows: CBD will inhibit *Id-1* and corresponding breast cancer metastasis in a mouse model. By making structural changes to CBD, we have potentially created compounds that have greater activity than CBD at inhibiting *Id-1* and corresponding breast cancer cell aggressiveness. Analogous of CBD, that have greater activity than CBD in culture, are expected to be more active than CBD at inhibiting tumor formation in mouse models of breast cancer metastasis. Experiments outlined in this proposal will lead to the discovery of the mechanisms involved in cannabinoid inhibition of *Id-1* and corresponding human breast cancer cell aggressiveness. Importantly, by understanding the mechanism through which CBD regulates *Id-1*, we expect to be able to synthesize future more active cannabinoid compounds. Objective: We will determine whether CBD inhibits *Id-1* and corresponding breast cancer metastasis. The ability of CBD and CBD analogs to inhibit aggressive breast cancer will be studied using specific assays that measure cancer cell growth and invasion in culture. In culture, the ability of cancer cells to invade is a measure of their metastatic potential. Those most active inhibitors discovered in culture will then be tested in mouse models of breast cancer metastasis. In cell cultures, biochemical techniques that can both measure and modulate protein expression will be used to discover mechanisms by which CBD inhibits *Id-1* and corresponding breast cancer cell aggressiveness. Patient advocacy and time line: The PI regularly meets with breast cancer patients and advocates during conferences and symposium. An important goal among breast cancer advocates is to find non-toxic therapies that specifically target metastatic breast cancer and not healthy tissues. Our targeted approach is expected to satisfy these criteria. Additionally, due to the difficulties in accessing efficient screening methods, metastatic breast cancer is more likely

Pending Execution of Grant Agreements

to be diagnosed in women with poor social conditions. Therefore, these new therapeutic modalities may particularly benefit undeserved populations with aggressive cancers. CBD is a novel compound by which the growth and spread of breast cancer may potentially be inhibited through down-regulation of Id-1.

We have outlined a strategy to create a family of breast cancer inhibitors that are even more active than the parent drug CBD. Additionally, we expect to discover the detailed mechanisms involved in cannabinoid inhibition of Id-1 and corresponding breast cancer cell aggressiveness. We are collaborating with a pharmaceutical company who is currently engaged in clinical trials testing the efficacy of CBD for indications unrelated to cancer. If CBD inhibits Id-1 and corresponding breast cancer metastasis in mouse models, there would be significant enthusiasm to move CBD toward clinical trials for the treatment of metastatic breast cancer. This process could be rapid since CBD is already being tested in the clinic for indication other than cancer and has an established safety profile. Our goal is to start clinical trials with CBD within three years. Our long-term goal is to follow up with second generation CBD analogs that are expected to be more potent and/or efficacious at inhibiting metastatic breast cancer in humans compared to CBD.

PI Name: Jong Yun

Mechanism: Investigator Initiated Research

Institution: Pennsylvania State University, College of Medicine

Application Title: Sphingosine Kinase Inhibitors as Anti-Breast Cancer Therapeutic Agents

Abstract:

Current treatments for metastatic breast cancer (BC) are not effective mostly due to the tumor's intrinsic or acquired resistance to available chemotherapeutic drugs. In fact, patients with the hormone-independent tumors have a poor prognosis as these tumors are closely associated with a higher rate of proliferation, metastasis and multi-drug resistance (MDR). Studies show that development and progression of breast cancers (BC) are closely associated with the altered expression of oncogenic proteins. Specifically, oncogene-induced BC cell activation stimulates BC cell growth, metastasis, and/or resistance to cell death. Sphingosine kinase 1 (SphK1) is a signaling lipid kinase that catalyzes the formation of pro-mitogenic (cell growth) sphingosine-1-phosphate (S1P) at the expense of pro-apoptotic (cell death) ceramide. Our studies show that SphK1 is significantly over-expressed in human breast tumor tissues compared to the adjacent normal tissues. Additional studies indicate that SphK1 is a key regulator of BC cell growth, invasion and survival. Importantly, a recent study of 1,269 breast tumor samples revealed a significant link between high SphK1 expression with metastasis and poor prognosis of patients. Based on this accumulating evidence, we believe blocking SphK1 activity would be an effective novel therapeutic approach to overcome multi-drug resistance and metastasis in BC. It is our hypothesis that metabolic conversion of pro-apoptotic (cell death) ceramide to pro-mitogenic (cell growth) S1P by SphK1 triggers BC cell growth. Therefore, blocking SphK1 activity should increase ceramide and decrease S1P, essentially "killing two birds with one stone", resulting in BC cell death. To date however, pharmacological inhibition of SphK1 is an untested means of treating BC. Hence, the primary goal of this study is to validate the therapeutic potential of targeting SphK1 in BC. To accomplish this goal, we proposed in vitro and in vivo studies that will critically evaluate the effectiveness of our novel SphK1 inhibitors (SKIs) as anti-BC therapeutic agents. These SKI lead compounds, identified by screening libraries of synthetic "drug-like" compounds, are highly cytotoxic toward BC cell lines including metastatic and/or MDR phenotypes. Additionally, our "proof-of concept" studies indicate that targeting SphK1 using our novel SKI compounds may be an effective approach in BC therapy. However, before we can propose preliminary human studies, we must optimize our SKI lead compounds to maximize the therapeutic index and minimize any undesirable side-effects. Hence, we propose in this study to refine our SKI lead compounds to improve the effectiveness of SKIs as anti-BC chemotherapeutic agents to overcome metastasis and/or to reverse MDR in BC. To accomplish these goals, we have developed synergistic interactions between basic scientists with expertise in cancer biology and chemists with expertise in bio-organic/medicinal chemistry. To validate the effectiveness of our "refined" SKIs, we designed our studies with specific experiments employing a number of synthetic, biochemical and pharmacological assays, cellular cytotoxicity assays, and in vivo animal studies. Findings from this study should provide insights into the improvement and/or development of a novel therapeutic approach that specifically targets SphK1 in BC including types that are metastatic (or ER-) and/or resistant (or refractory) to current treatments.

PI Name: Shyamala Maheswaran

Mechanism: Investigator Initiated Research

Institution: Massachusetts General Hospital

Application Title: Functional consequence of deregulated HoxB9 expression in breast tumors

Abstract:

Hox genes are proteins that play an important role in tissue development. They are misexpressed in several types of cancer including breast cancer. However, the functional consequence of misexpressed Hox in promoting tumorigenicity in the breast, or misexpressed Hox as a possible marker for selecting patients for molecularly targeted therapy to reduce breast cancer mortality have not been well explored. Our results show that HoxB9, a Hox gene, is overexpressed in 42% of human breast carcinomas; overexpression correlated with higher tumor grade. Experiments carried out in mice show that HoxB9 overexpressing breast tumors are larger in size, exhibit increased blood supply, and metastasize to the lung. At a molecular level, HoxB9 induces several proteinacious factors that are secreted into the tumor microenvironment. These proteinacious factors have been shown to increase blood supply to the tumor and induce metastasis. The overall goal of this grant is to test whether suppressing the induction of these proteinacious factors can block the growth, blood supply and metastatic potential of HoxB9 overexpressing breast tumors and thus reduce breast cancer mortality. We will also test whether the stem-like properties acquired by the HoxB9 expressing breast tumor cells will render them resistant to radiation and chemotherapy.

Clinical Impact and the potential to reduce metastasis and mortality: HoxB9 is overexpressed in 42% of human breast cancers. The purpose of this grant is to understand the functional consequence of HoxB9 overexpression in breast cancer progression, with the ultimate translational goal of determining whether tumoral overexpression of HoxB9 could serve as a biomarker to predict a robust response to targeted therapy with inhibitors against proteinacious factors which (1) promote tumor growth, (2) increase blood supply to the tumor and (3) induce metastasis to the lung. We propose that these targeted therapeutic choices will suppress the metastasis of HoxB9 overexpressing tumors and thus decrease breast cancer mortality.

The experiments will also help us determine whether HoxB9 overexpression induces stem-like properties in tumors and hence confer resistance to conventional radiation- and chemotherapy. In the current clinical setting, molecularly targeted therapies are greatly desired and are considered as state-of-the art treatment for cancer patients. Therefore, the experimental outcome of this grant will have tremendous impact in exploring the possibility of protecting patients from unnecessary toxicities of ineffective therapies and identifying patients for targeted therapy to decrease blood supply to the tumor and block distal metastasis. By targeting the reduction of metastatic disease progression it will directly impact breast cancer mortality and increase survival, a goal that fits well with the mission of the Susan G. Komen for the Cure.

PI Name: Jennifer Richer

Mechanism: Investigator Initiated Research

Institution: University of Colorado Health Sciences Center

Application Title: Restoration of MicroRNA-200c: a Novel Differentiation Therapy for Treatment of Aggressive Breast Cancers

Abstract:

For years there have been regions of the genome that were not thought to have a function, and were in fact referred to as 'junk DNA'. Recently, however, a new class of molecules has been discovered called microRNAs. It turns out that many of the regions of 'junk DNA' aren't junk at all - they code for these microRNAs. Each microRNA controls the expression of a number of specific proteins and as such, can have very potent effects on cells. One of the features of aggressive breast cancer is that the cancer cells change their identity and begin to act in a way uncharacteristic of normal breast cells. We have found that a particular microRNA, microRNA-200c, is an important controller of breast cell identity. We find that microRNA-200c is expressed in breast cancer cells that are less aggressive and retain much of their breast cell identity. However, in highly aggressive breast cancer cells, there is a loss of microRNA-200c. Our preliminary data shows that re-introduction of microRNA-200c into highly aggressive cancer cells causes them to regain some of their normal cell characteristics. Importantly, it renders them less migratory and more sensitive to certain anti-cancer treatments.

We hypothesize that re-introduction of microRNA-200c into aggressive breast cancer cells will be make them act more normal, rendering them less metastatic and more sensitive to treatments.

To test our hypothesis, we have designed three specific aims. In the first aim, we will use a system in which we can turn microRNA-200c on and off. We will implant breast cancer cells into mice and turn microRNA-200c on before metastases form to determine if it can prevent metastases. We will also turn microRNA-200c on after metastases have formed to determine if it can cause regression of already established metastases. Since we have found a correlation between loss of microRNA-200c and aggressiveness in breast cancer cell lines, in the second aim of our study, we will look at clinical samples, including the most aggressive and least treatable forms of breast cancer, to determine if the same correlation holds true and to determine the mechanism whereby the microRNA is lost. Our preliminary data has shown that re-introduction of microRNA-200c to aggressive cancer cell lines makes them more sensitive to certain chemotherapeutics. In our third aim, we will use mouse studies and clinical samples to determine if these samples will also become sensitive to chemotherapeutics when microRNA-200c is re-introduced.

The field of microRNA research is very new and the proposed studies use cutting edge technologies. There are relatively few published reports of the involvement of microRNAs in breast cancer; however initial studies implicate microRNAs as having an important role. In fact our own preliminary data implicates microRNA-200c as having a central role in breast cell identity, invasive potential and sensitivity to chemotherapeutics giving it great potential as a new form of therapy, or at the least, potential to greatly increase the efficacy of traditional chemotherapy.

Pending Execution of Grant Agreements

Our proposed studies have high potential impact and potential to improve treatment for a subset of patients for which there is not currently any form of targeted therapy option: the aggressive so called “triple negative” breast cancers and others termed metaplastic and micropapillary breast cancers . Furthermore, among racial/ethnic groups, African Americans are significantly more likely to be diagnosed with these aggressive types of tumors, which are most likely to benefit from this type of therapy. Restoration of miR-200c is a type of therapy in which we will attempt to have cancer cells to revert back to a more normal state, thus we anticipate that this type of therapy will have few toxic side effects. The proposed studies are the first steps towards using microRNA-200c as a novel therapeutic to prevent or treat metastatic breast cancer. If our pre-clinical studies work, it should not take long to move this type of therapy to the clinical trial setting.

PI Name: Matthew Ellis

Mechanism: Investigator Initiated Research

Institution: Washington University in St. Louis, School of Medicine

Application Title: Whole Genome Profiling and Functional Genomics in Breast Cancer

Abstract:

General Audience Summary: Breast Cancer is a complex disease with patients experiencing very different fates. Some are cured with surgery alone and others suffer repeated relapses of their disease despite all available treatments. One of the major reasons for these very different outcomes is that at the DNA level, breast cancers are remarkably diverse. New breakthroughs in cancer genome analysis, including sequencing entire cancer genomes, are currently being implemented that will document this diversity. Current evidence suggests that the constellations of gene changes may be unique to every cancer. This complexity presents an extraordinary challenge as we try to determine which gene changes can be translated into new approaches to breast cancer prevention, diagnosis, and treatment. Hypothesis: Whole genome tumor profiling and clinical data linked to “Human in Mouse” tumor lines can be used to assess the therapeutic significance of novel findings in the breast cancer genome.

With initial support from the Susan G Komen Foundation, we have demonstrated the feasibility of a comprehensive and innovative approach to breast cancer genomics termed “Human and Mouse Linked Evaluation of Tumors (HAMLET).

In these studies, patients donate several specimens from their breast cancer to be analyzed in detail for cancer-specific changes in the patterns of gene activity and for the presence of cancer-specific changes in the tumor DNA. A portion of these specimens are also engrafted into a special breed of mice that does not have an active immune system in order to allow the human tumor to grow. These tumor lines can be subsequently transplanted from mouse to mouse to test innovative treatments and for detailed studies on the biological significance of the gene changes found in the human tumor.

In the first year of an initial two year grant from the Susan G. Komen Foundation we have successfully established eight tumor lines from seven patients and have found that a) the engrafted tumors are biased towards highly lethal basal-type tumors occurring in African Americans and b) morphological, genomic, biomarker and somatic mutation characteristics are remarkably well preserved in the grafting process. We are in the process of leveraging our research through multiple collaborations to address a series of innovative therapeutic, genetic and biomarker hypotheses in the HAMLET system. In our new proposal we will continue careful and detailed experiments to increase the number of validated HAMLET tumor lines. We will pilot a system of global tumor acquisition and distribution of successful HAMLET grafts so that this important research technique can be widely distributed. Finally we will show that each tumor line can be tagged with a green fluorescent marker to increase the efficiency of therapeutic experiments and to demonstrate that genetic manipulation of the HAMLET tumor lines is feasible.

If we are successful in this project we envisage a remarkable acceleration in breast cancer research because we will be more confident that preclinical mouse findings are directly relevant to the clinical realities our patients face. Examples of the potential utility of the HAMLET research approach include: 1) the ability to systematically work through the DNA

Pending Execution of Grant Agreements

changes to work out which ones are critical to tumor formation; 2) new insight into why tumors are sensitive or resistant to standard treatment; 3) a better system for testing new agents and approaches pre-clinically so novel therapeutic strategies have a higher success rate when tested in human clinical trials.

PI Name: James McIntyre

Mechanism: Investigator Initiated Research

Institution: Vanderbilt University, School of Medicine

Application Title: Real Time Assessment of Self-Reporting Chemotherapeutics for Targeted Treatment of Metastatic Breast Cancer

Abstract:

(1) Hypothesis and how it will be tested: We hypothesize that the development of a water-soluble, detectable, nanoparticle proteinase-activated paclitaxel (PXL) prodrug (ND1-PXL) will specifically target PXL delivery to breast cancer metastases increasing therapeutic efficacy while reducing toxicity (particularly nervous system toxicity). Testing the hypothesis: ND1-PXL will first be tested and refined using established mouse models of breast cancer, then as rapidly as possible transitioned to VICC for clinical testing, Peripheral neuropathy (neurotoxicity resulting in either pain and/or loss of sensation in the limbs) is a major debilitating complication of PXL or albumin-PXL (Abraxane or nab-PXL) therapy. The new ND1-PXL drug is designed to be water-soluble and less neurotoxic than either of the current formulations. A set of neurobehavioral studies will be carried out in mice to assess potential neurotoxicity of ND1-PXL versus nab-PXL so as to test this postulate. Components of the delivery vehicle of ND1-PXL will be modified as necessary to reduce any manifest neurotoxicity. Death from breast cancer is directly related to the ability of tumor cells to metastasize throughout the body. Treating patients who have primary breast cancer with chemotherapy adjunctive to surgery is beneficial, supporting the idea that "seeking and destroying" cancer metastases at their very earliest stages can prolong life. Current therapies have unwanted side effects because they don't just target tumor cells, but kill any cell with rapid division. One approach to make more effective but less toxic chemotherapy drugs for the treatment of breast cancer and metastatic disease is to make "prodrugs" that are toxic only after biological processing at the tumor target. In our approach, we target chemotherapy by harnessing protease activities prevalent in the vicinity of tumors and tumor metastases (the tumor microenvironment). We have designed a prodrug, ND1-PXL, that is activated by MMP9, a matrix metalloproteinase prevalent in the tumor microenvironment. This approach is independent of the biological effect of the MMP itself; thus, the well-known failure of systemic MMP inhibitors in clinical cancer trials is irrelevant in this context. In the presence of MMP9 activity, ND1-PXL will selectively deliver a toxic dose of PXL to the tumor. Protease-activation has the added advantage that multiple ND1-PXL molecules can be activated by a single protease molecule, providing amplification of response as compared with receptor-targeted agents. ND1-PXL and the bifunctional ND1-ND2 prodrugs will be used to "seek and destroy" both primary and metastatic breast cancer and to "self-report" delivery of the targeted therapy while minimizing the systemic side effects associated with standard chemotherapy. We expect to achieve highly targeted delivery of the cytotoxic drug to tumor cells with appropriate controls and safeguards for delivery and treatment response.

(2) Potential for reducing mortality from breast cancer: Nanotechnology allows us to design and test sophisticated new prodrugs with potential for enhancing the detection of small metastatic nodules and to more selectively kill metastatic breast cancer cells while sparing normal cells. The therapeutic efficacy will be tested in mouse models of breast cancer metastases and the prodrugs modified as necessary to achieve detection and targeted

therapy of primary tumors and lung metastases. The successful testing of these prodrugs in the mouse models of breast cancer metastasis will lead immediately to Phase I clinical trials in breast cancer patients. For such clinical trials, the targeted prodrugs will first be tested in patients previously treated for metastatic breast cancer; first to explore toxicity (maximum tolerated dose) and then to evaluate therapeutic potential in patients unresponsive to first-line therapy as appropriate for their tumor status (i.e., ER+, HER2+, etc). The goal of the clinical trials will be to extend and improve the quality of life for breast cancer patients that have either clinically detected or occult metastatic disease. Our ND1-PXL prototype and subsequent variations developed on this novel targeted therapeutic platform have significant potential to reduce mortality in breast cancer patients within the next decade or sooner.

(3) Importance of research to patients with breast cancer: Metastatic disease and the recurrence of breast cancer are the major life-threatening complications of breast cancer. The goal in developing, testing and improving, as necessary, the tumor-targeted therapeutic agents we have devised is to provide drugs that will selectively eliminate small clusters of cancer cells that may have metastasized (migrated to distant sites in the body) at time of first diagnosis. After the planned pre-clinical validation of efficacy and safety of the new ND1-PXL, Phase I clinical trials of our new tumor-targeted drugs in breast cancer patients will follow immediately with the goal to extend and improve the quality of life for breast cancer patients that have either clinically detected or occult metastatic disease. We anticipate that the use of these kinds of targeted prodrugs designed to eliminate metastatic disease by targeting molecular components associated with the microenvironment of tumor metastases will improve the treatment of breast cancer, particularly metastatic disease. In particular, this reagent and other drugs built on this novel platform will facilitate custom-designed approaches (personalized medicine) to detect and treat breast cancer micrometastases particularly for the node-negative breast cancer patient.

PI Name: Jihe Zhao

Mechanism: Investigator Initiated Research

Institution: Albany Medical College

Application Title: Determining Role of EMT in Metastatic Progression of Human Breast Cancer.

Abstract:

METASTASIS REMAINS THE MAIN CAUSE OF DEATH FROM HUMAN BREAST CANCER. One out of every 8 women in their lifetime will develop breast cancer. Each year in the US, metastatic breast cancer kills 1 woman every 13 minutes and every 3 minutes a new case of invasive breast cancer is found, despite significant advances made in the realms of cancer prevention, detection, and management of primary breast tumors. This is primarily due to the failure of effective detection and management of breast cancer metastases. Consequently, the chance for a patient to live for 5 more years after diagnosis falls from > 90% for localized diseases to < 20% once metastasis has occurred. Hence, the only way to improve the patient survival is to prevent the cancer from metastasis and understanding the molecular mechanisms responsible for the metastasis is the key.

BREAST CANCER METASTASIS starts from the primary tumor that originates from the mammary gland epithelial cells. From there, the cancer cells find their ways to invade the surrounding matrix tissues (dissemination) and to enter the blood or lymph streams (intravasation), to survive the streams as circulating tumor cells (CTC), to exit the circulation through the vessel walls (extravasation), and to form secondary tumors in distant vital organs such as the lungs and bones (colonization). This whole process is known as tumor metastasis. In the beginning of dissemination, the invasive tumor cells leave the primary tumors by a mechanism known as EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) that disrupts the interaction between the tumor cells and provides the cells with enhanced ability to migrate away from the localized tumor and to invade through the surrounding tissues. The dissemination, intravasation and extravasation require the invasive tumor cells to create such an extracellular environment that allow enzymes, called proteases such as MMP (Matrix metalloproteinase) and PA (plasminogen activator) proteins, to degrade the matrix proteins particularly in the vessel's basement membranes. During the travel in the circulation, the CTCs usually minimize or stop proliferating activity and must survive death threats and destruction by circulating killer leukocytes. It is believed that the CTCs that make their way out of the circulation can be dormant for up to 20 years after colonizing in the distant organs before onset of the secondary tumor growth. The secondary tumor growth requires the dormant cells to resume proliferation and angiogenesis. Given the multi-step nature of the metastatic process, opportunities exist for early diagnostic, prognostic and therapeutic targeting of the metastatic cells before clinical problems arise. Better understanding of the molecular mechanisms behind each of the steps is the key to secure the opportunities.

Since EMT occurs in the very early phase of tumor metastasis, targeting EMT inducing proteins should be an effective approach for metastasis prevention. The defining event for EMT is disruption of cell-cell interaction through a linker protein called E-CADHERIN. The loss of E-cadherin leads to separation of tumor cells from one another that gain drastically increased ability to migrate and invade (i.e., to spread). Loss of E-cadherin along with

Pending Execution of Grant Agreements

elevated MMPs (protein degrading enzymes known to be primary contributors to breast cancer metastasis) levels has been found in the vast majority of invasive breast cancer, strongly suggesting the possibility that EMT is critical to breast cancer metastasis.

We have found that Krüppel-like factor 8 (KLF8), a protein serving as a switch that controls the production of other proteins, potently induces EMT and invasion in human breast cells by inhibiting the production of E-cadherin and increasing the production/activity of MMP9, MT1-MMP and MMP2. We have also found that in invasive breast cancer cells and tumor tissues collected from human patients, KLF8 levels are significantly elevated and this elevation plays a large part in the loss of E-cadherin and gain of the MMPs. These novel findings prompt us to further determine the role and molecular mechanisms for KLF8 in cancer metastasis using in vivo models that truly mimic the pathology of human breast cancer metastasis. INDEED, OUR NEW DATA SUPPORT A CRITICAL ROLE FOR KLF8 IN MDA-MB-231 LUNG METASTASIS.

We have assembled all the tools necessary for the proposed studies. This project aims at extending our novel findings with the near-future goal of translating the lab discoveries into clinical application to treat and prevent breast cancer metastasis and improve the patient survival. Since we have demonstrated that KLF8 upregulation in cancer results from its elevated transcription, inhibitors specific for KLF8 transcription would become likely therapeutic agent for KLF8-targeted treatment and prevention of breast cancer metastasis. ESTABLISHING OUR PROPOSED SCREENING TOOLS FOR KLF8 INHIBITORS WILL MAKE ONE STEP CLOSER TO THE SUSAN G. KOMEN'S GOAL TO REDUCE BREAST CANCER INCIDENCE/MORTALITY WITHIN THE NEXT DECADE.

PI Name: Ratna Vadlamudi

Mechanism: Investigator Initiated Research

Institution: University of Texas Health Science Center at San Antonio

Application Title: Role of PELP1 in local estrogen synthesis and breast cancer progression

Abstract:

Estrogen receptor is implicated in breast cancer progression. Aromatase, a key enzyme involved in estrogen synthesis is expressed in breast tumors and locally produced E2 might act in a paracrine or autocrine fashion. In recent years, hormonal driven tumors have been targeted with aromatase inhibitors, such as letrozole, in an adjuvant setting. Accumulating evidence also suggest that a variety of different factors may regulate expression and activity of aromatase under pathological conditions and local production of estrogen may enhance tumor growth and may also interfere with hormone therapy resistance. Therefore, better understanding the signaling pathway (s) that contributes to local E2 synthesis in tumor cells is needed for development of alternative strategies.

Emerging evidence suggest that histone methylation, an epigenetic phenomena, could play a vital role in many neoplastic processes by silencing and activation of tumor suppressors and oncogenes respectively, and thus represents a valuable therapeutic target. Estrogen-induced breast carcinogenesis is shown to be characterized by alterations in histone modifications. Recent studies showed that demethylase enzyme LSD1 is recruited to a significant fraction of ER target genes and is shown to be required to demethylate proximal histones to enable ER-mediated transcription. These emerging findings also suggest that deregulation of this epigenetic pathway could lead to hormonal independence and therapy resistance.

The rationale for the study emerges from recent studies in our laboratory that PELP1 (Proline, Glutamic acid, Leucine rich Protein 1), a novel ER coregulator, functions as a potential proto-oncogene, modulates epigenetic changes at ER target genes and aromatase gene and its expression is deregulated in breast tumors. Our preliminary studies suggest that PELP1 deregulation contribute to local E2 synthesis and cooperates with growth factor signaling components in the activation of the aromatase. The central hypothesis is that deregulation of PELP1 signaling and its interactions with growth factor signaling components, epigenetic modifiers, influence the in situ production of E2, leading to tumor progression and hormone independence.

The hypothesis will be tested using two specific aims; (1) To establish the role of PELP1 in regulation of aromatase and breast cancer progression. (2) To investigate the significance of PELP1-LSD1-axis on the breast tumor initiation, progression and hormonal therapy resistance.

To accomplish these goals, we will use novel breast model cells that overexpress ER-coactivator (PELP1) or breast oncogene neu/HER2 and novel Tg mice model that overexpress PELP1 in the mammary gland. In Aim1, we will determine the role of PELP1 in tumor progression and establish the molecular mechanism by which PELP1, LSD1 regulate aromatase expression in breast tumor cells. In Aim2, we test the therapeutic potential of blocking PELP1-LSD1 axis in the initiation, progression of breast tumor by blocking PELP1 using nano-scale PELP1-siRNA liposomes and by using LSD1 inhibitor (Pargyline), alone or in combinations with aromatase inhibitors using in vitro and in vivo pre clinical models.

Pending Execution of Grant Agreements

Further, this study will also test the ability of Pargyline an FDA approved drug for hypertension to restore hormonal sensitivity in resistant model cells. At the completion of this project it is our expectation that we will have identified a novel pathway that contribute to in vivo E2 synthesis and test the ability of novel drugs that target PELP1-LSD1 axis to block E2 synthesis. Since In situ estrogen synthesis is implicated in breast tumor cell proliferation, combination therapies that block aromatase activity as well as agents that block local aromatase expression may have better therapeutic effect and may delay development of hormonal resistance and open a new avenue for ablating local aromatase activity in endocrine therapy-resistant breast tumors. This research is clinically significant as it will test the in vivo therapeutic potential of PELP1-LSD1 axis using emerging nanotechnology and siRNA therapeutics and by using Pargyline, an FDA approved drug for hypertension. Generation of drugs that affect PELP1 will have potential use in reducing progression and development of resistance in breast cancer. In addition, our proposed combination therapies targeting hormonal signaling axis using letrozole, PELP1 nanoliposomes, paragyline will have better therapeutic effect and may delay development of hormonal resistance thus could provide major benefits to patient's care.

PI Name: Celia Kaplan

Mechanism: Investigator Initiated Research

Institution: University of California at San Francisco

Application Title: Breast Cancer Risk Reduction in Primary Care Clinics: A Bilingual Intervention for Women and Physicians

Abstract:

Public Abstract During the last decade, new knowledge and technologies within the field of breast cancer research have become available which have led to the recommendations to include breast cancer risk assessment in primary clinical care. Health information technology (IT) tools (computer-generated aids) have been shown to promote positive outcomes in patient health and care, providing women with the means to obtain assessments of breast cancer risk and critical breast information in an easy, and convenient manner. Despite advances in both areas of research, many women remain unaware of their individual risk, and physicians have been unable to integrate risk assessment and discussion into their routine practices due largely to time constraints. The end result is that breast cancer risk assessment tools, as well as discussion and use of prevention and risk reduction therapies, have been greatly underutilized. Without timely assessment, breast cancer may be detected at a later stage, requiring more invasive treatments, or worse, detected too late for viable treatment.

The proposed research combines the scientific advances in breast cancer research with health IT to design an appropriate intervention to assess breast cancer risk, disseminate important breast health information, and provide a vehicle to reduce incidence of and mortality from the disease. Our goal is to implement a tablet PC-based breast cancer risk education (BCRE) intervention in the primary care setting that estimates a woman's individual risk for breast cancer and provides her and her physician with personalized breast cancer risk information and recommendations for action.

STUDY HYPOTHESIS: The proposed study evaluates a tablet PC-based breast cancer risk education intervention in a primary care setting. The objectives of the intervention are to a) increase patient knowledge about her individual risk of developing breast cancer, b) increase discussion of breast cancer risk reduction practices based on individual risk, and c) ensure up-to-date mammography screening.

Two hypotheses will be tested: 1) women in the BCRE intervention group will indicate high rates of satisfaction with the format and content of the intervention, and 2) women in the BCRE intervention group, as compared to those in the control condition, will report increased knowledge of risk reduction options, accurate perception of own risk, greater participation in patient-physician discussion of breast cancer risk and recommendations of risk reductions based on patient risk, and more up-to-date mammography screening.

ADVANCEMENT OF UNDERSTANDING: Our intervention has the potential to reduce the incidence of breast cancer by providing each woman with accurate information about her individual risk of breast cancer and risk reduction and prevention therapies in a format that is easy to understand and readily available at her primary care clinic. It is an educational tool that promotes risk-reducing therapies (e.g., chemoprevention), appropriate referrals (e.g., referral to genetic counseling), and known risk reduction health behaviors (e.g., lowering alcohol

Pending Execution of Grant Agreements

intake). In addition, it monitors and encourages mammography screening at a time when screening rates among women are in decline.

PATIENT IMPACT: At the clinic setting, the tablet PC-based breast cancer risk education intervention will improve health care delivery for all women. Primary care physicians rarely engage in breast cancer risk assessment or discuss risk reduction options, even when patients are at high-risk, primarily due to time constraints faced by physicians operating in busy practices with competing demands. Although well-established tools for risk assessment and expanding options for risk reduction are available, they are not optimally integrated into primary care practice, resulting in a missed opportunity to prevent breast cancer and reduce mortality due to the disease. Our intervention will provide a time-efficient, systematic strategy to bring breast cancer risk reduction at the forefront of care. Since our intervention will address the needs of Spanish-speaking women, it is likely to have an even greater impact on Latinas who face language barriers that preclude them from receiving accurate health information and who have less ability to discuss risk than their English-speaking counterparts. The intervention will help encourage Spanish-speaking women to play a more active role in their health and participate more in decision-making. In sum, our application of this health IT tool has the capacity to help women understand their risk of developing breast cancer, inform and provide them with the therapeutic options to prevent the disease, and improve communication between patients and physicians about breast cancer topics. It also promotes patient-centered care, empowering women with personalized information based on their individual risk.

PI Name: Toshihiro Shioda

Mechanism: Investigator Initiated Research

Institution: Massachusetts General Hospital

Application Title: Systematic RNAi Knockdown and Systems Biology Approach Reveal Roles and Interactions of Signaling Pathways in Mechanisms of Acquired Antiestrogen Resistance

Abstract:

Approximately 70% of breast cancers express estrogen receptor protein. Most of the tumors that express this marker protein is dependent on estrogen for their growth and survival, as long as the tumors are restricted at its original location in the breast without metastasis. The aim of endocrine therapy of breast cancer is to shut off the estrogen signaling in the cancer cells.

Although the advent of endocrine therapy revolutionized the treatment of breast cancer, its effectiveness is seriously limited by drug resistance. After metastasis, about 50% of estrogen receptor-positive tumors no longer respond to endocrine therapy. Even when metastatic tumors initially respond to endocrine therapy, practically all these tumors will eventually develop drug resistance. Approximately 40% of early-stage breast cancers treated with pre-surgery tamoxifen will relapse with tamoxifen-resistant tumors. Effectiveness of aromatase inhibitors, the latest arsenal of endocrine therapy agents, are also limited by drug resistance of tumors. Thus, elucidation of mechanisms of endocrine therapy resistance is urgently desired.

A large number of published studies have proposed a wide variety of molecular mechanisms of endocrine therapy resistance of human breast cancer cells. One of the most important recent discoveries accomplished in this research field is that the cross-talk between the intracellular cascade of events activated by estrogen and other lines of events activated by alternative stimuli seems to play critical roles in determination of breast cancer cell sensitivity to endocrine therapy.

The traditional process to integrate a large number of individual pieces of scientific information obtained from original publications into systematic knowledge has been dependent on discussions and communications among investigators through meetings and publications of review articles or textbooks. This process is slow, and it may significantly delay practical clinical outcome from the basic studies on breast cancer therapeutics. However, if we can immediately obtain a comprehensive map of molecular interactions that depicts many proteins and their mutual interactions occurring in human breast cancer cells and being relevant to resistance to endocrine therapy, such a map will dramatically facilitate formation of the integrated understanding on the mechanisms of drug resistance in the scientific community. A scientist whose work focuses on one or a few proteins can use the map as a guide to identify the position of his/her study in the large picture of molecular network that is responsible for determination of sensitivity to endocrine therapy and to select the future focus of studies for the best effectiveness to fight breast cancer.

Experimental generation of the molecular interaction map within a reasonable period of time (in a couple of years) has been recently made practically feasible through the advancement of high-throughput laboratory automation technology and computer-aided prediction of molecular interactions. The principal investigator of this proposal has been operating a

central technology resource facility for these innovative technologies at the Massachusetts General Hospital (MGH) Center for Cancer Research, an internationally recognized research institute. As Director of the facility, he has strong expertise in these key technologies necessary to build a comprehensive molecular interaction map. In addition, he also holds publication-proven expertise in research on molecular mechanisms of antiestrogen actions in human breast cancer cells. Using the RNAi knockdown screening technology that identifies proteins required for antiestrogen sensitivity of cultured breast cancer cells, and a cutting-edge computational tool to predict molecular interactions, our project aims to develop an evidence-based molecular interaction map that depicts relationships among a large number of proteins responsible for determination of sensitivity of breast cancer cells to endocrine therapy.

The map thus developed using a breast cancer cell culture model will be then validated through the established laboratory testing on a large collection of breast cancer tissue samples archived in the MGH Breast Pathology Program. The Director of this pathology program will serve as the Co-PI of this research project. The pathological analyses will not only bridge the knowledge obtained from a cell culture model to human disease, but also examine whether patients with different ethnic backgrounds have different molecular mechanisms of endocrine therapy resistance.

This project also has significant potential to lead to reductions in breast cancer mortality within the next decade. The molecular interaction map generated in this project may provide physicians with critical insights into how clinically practical strategies to simultaneously control multiple avenues of molecular events in breast cancer cells, in an attempt to suppress endocrine therapy resistance. Using clinically proven therapeutic agents that are already approved by FDA for other types of malignancies or diseases, physicians may be able to launch clinical trials for such novel, combinatorial drug therapies very quickly. In fact, based on the limited, presently available information on mechanisms of endocrine therapy resistance, several clinical trials for such combinatorial therapies have already been initiated. Thus, our research project is not only important for future scientific understanding of breast cancer biology, but also has promise to be practically helpful for the present breast cancer patients to fight this disease.

PI Name: Marcelo Kazanietz

Mechanism: Investigator Initiated Research

Institution: University of Pennsylvania, School of Medicine

Application Title: P-Rex1 in ErbB Signaling and Breast Cancer

Abstract:

ErbB receptors are surface proteins present in mammary cells (and other cells) that promote cell division and cell survival. When some of these receptors are hyperactive or present at abnormal high levels, a normal mammary cell can become cancerous. For example, mutations in EGFR or very high ErbB2/HER2 (both members of the ErbB receptor family) are common genetic alterations in breast cancer cells. It is highly important to understand the nature of the signals emanating from ErbB receptors and how they contribute to the formation of breast tumors and metastasis for many reasons. First, ErbB receptors have significant therapeutic relevance as they are the targets of well-established therapies, such as Herceptin or other antibodies and small molecule inhibitors. Second, ErbB receptors are important prognostic markers for breast cancer (for example ErbB2/HER2). Third, several of the intracellular effectors of ErbB receptors are dysregulated in breast cancer (for example, the PI3K/PTEN pathway). A better understanding of these pathways should help improving the efficacy of current therapeutic approaches.

The main objective in this application is to address the relevance of a protein called P-Rex1 in human breast cancer. P-Rex1 activates Rac1, a protein that is crucial for cell motility, tumorigenicity and metastatic dissemination of cancer cells. Indeed, pharmacological or genetic inhibition of Rac1 impairs tumor formation and metastasis. Emerging evidence indicates that Rac1 is hyperactive in breast cancer and that this correlates with aggressiveness of the tumor and resistance to the killing effect of anti-estrogens such as tamoxifen. Therefore, the identification of P-Rex1 opens a new avenue to “manipulate” Rac signaling and potentially inhibit breast cancer progression and improve the therapeutic efficacy of anti-estrogens.

We have made two fundamental discoveries. First, we identified P-Rex1 as a mediator of Rac1 activation in response to stimulation of ErbB receptors in breast cancer cells. Second, we found that although P-Rex1 is almost undetectable in normal mammary cells, it is unusually high in human breast cancer. Therefore, P-Rex1 may mediate tumorigenic and metastatic effects driven by ErbB receptors in breast cancer cells.

We propose to address the relevance of P-Rex1 in breast cancer using multiple approaches, including cell culture models, animals, and human breast cancer specimens. We will engineer breast cancer cells that normally express high P-Rex1 levels to lose P-Rex1 using a technique called RNA interference (RNAi). We predict that P-Rex1-depleted breast cancer cells lose their ability to migrate and proliferate in response to ErbB2 overexpression or ligands of the ErbB receptors, such as heregulin, a growth factor that is highly expressed in breast tumors and that confers an aggressive phenotype. We also expect that reducing P-Rex1 levels will overcome the resistance of breast cancer cells to anti-estrogens such as tamoxifen.

To address the relevance of P-Rex1 in vivo, we will inoculate P-Rex1-deficient breast cancer cells in mice and determine whether these cells lose their ability to form tumors and/or metastasize. We will also generate the first transgenic mouse model with high P-Rex1 levels

Pending Execution of Grant Agreements

specifically in the mammary gland. The prediction is that transgenic P-Rex1 overexpression (as we observe in human tumors) causes mammary pre-neoplastic lesions in mice and/or synergizes with other genetic alterations, such as PTEN deficiency, to promote breast cancer.

Lastly, we will pursue a rigorous analysis of P-Rex1 expression levels in breast tumors, taking advantage of a large collection of samples available to the P.I. These studies will allow us to determine whether P-Rex1 levels correlates with disease stage, outcome, and clinicopathological features of the disease, such as ErbB2/HER, estrogen-receptor (ER), and progesterone receptor (PR) status.

The proposed research is highly innovative, since it deals with a previously unappreciated paradigm in breast cancer. In summary, we anticipate learning novel fundamental concepts that should directly impact on our understanding of the molecular basis of the disease and therefore serve as the foundation for novel therapeutic avenues, as follows: a. Our research should have great impact on untangling signaling networks that trigger breast tumorigenesis and breast cancer cell metastatic dissemination.

b. Conceivable, it may possible that targeting the P-Rex1/Rac1 pathway in breast cancer impairs tumor formation and metastatic dissemination of breast cancer cells.

c. A second therapeutic implication is that drugs that inhibit ErbB receptors may conceivably lead to the inactivation of P-Rex1 and Rac to promote their beneficial therapeutic effects.

d. A third therapeutic implication is that our studies may underscore a novel mechanism by which breast cancer cells are resistant to anti-estrogens, a major problem faced by breast cancer patients, and hopefully it could be possible to translate these findings into the therapeutic arena by inhibiting P-Rex1 signaling.

e. Finally, our research may reveal a novel prognostic marker for breast cancer. Based on the well-established role for Rac in malignant transformation and metastatic dissemination, it is possible that P-Rex1 levels represent a novel biomarker that predicts aggressiveness of breast cancer tumors and metastatic dissemination.

PI Name: Irene Wapnir

Mechanism: Investigator Initiated Research

Institution: Stanford University, School of Medicine

Application Title: From Bench to Bedside: Treatment of Breast Cancer Brain Metastasis with ¹³¹I and Radiosensitizers

Abstract:

Breast cancer-brain metastases are becoming more common and are predominantly estrogen/progesterone-receptor negative (hormone independent). Tumor spread to the brain is an ominous sign associated with poor survival. Surgery and radiation therapy have limited efficacy. Systemic therapies have far less activity in the central nervous system than in other sites because the blood-brain barrier impedes entry of most anticancer drugs. However, it may be possible to make use of a natural occurring protein in breast cancer cells, the iodine transporter called NIS, as a delivery vehicle for radioactive iodine. While this concept is new in breast, it has been used extensively for the treatment of thyroid cancer since the 1940's. Moreover, the same mechanism is useful for imaging purposes, permitting the visualization of the tissue harboring NIS. Thus, NIS has a dual role as a diagnostic tool and as a predictive marker of tumor response to radioactive iodine.

The objective of this application is to test radioactive iodine as a new therapy with breast cancer-brain metastases in an experimental animal model and also determine whether drugs that heighten tumor cell radiosensitivity enhance its effect. Our secondary objective is to implement a small pilot treatment study in women with brain metastases. We hypothesize that breast cancer-brain metastases are treatable with radioactive iodine when the sodium iodide symporter, NIS, is present and active. In order to perform the necessary animal experiments, we have engineered human breast cancer (hormone independent, MDA-MB-231 and SKBR-3) cell lines to produce NIS, and have used them to develop brain metastases models in mice. Moreover, a second gene that produces a protein capable of emitting luminescence was linked to the NIS gene so that the growth of tumors and response to treatments can be tracked by a special imaging camera. Briefly, tumor cells are injected into the mammary gland area of immune-deficient mice and after tumors develop, small fragments are then transplanted into the brain of the same mouse. Using this established model we now propose to investigate: (I) how much iodine is concentrated by brain metastases and how well radioactive iodine (¹³¹I) destroys these tumors; (II) determine if tumor response is greater to combined of radioactive iodine and one of 3 drugs (lapatinib, gefitinib and gemcitabine) known to enhance radiation sensitivity; and (III) carry out a pilot treatment study of women identified by iodine-124 PET/CT imaging to have iodine-accumulating brain metastases. This project is a comprehensive effort to address important questions regarding the selective delivery of radioactive iodine therapy to breast cancer brain metastases. Based on prior clinical studies, normal thyroid tissue uptake of radioactive iodine can be effectively blocked with thyroid medications without compromising the therapeutic effect on breast cancer cells. These studies will advance our understanding of NIS-based radioactive iodine treatment and introduce an alternative and novel strategy for patients who carry a dismal prognosis. NIS-based treatment is a tailored, noninvasive, relatively inexpensive, individualized therapy with associated minimal toxicity. These features alone make NIS-based radioactive iodine treatment of breast cancer-brain

Pending Execution of Grant Agreements

metastases a compelling approach. If successful, radioactive iodine could reverse some of the debilitating neurological and cognitive side effects inherent in the presence of brain metastases, halt the progression of disease and possibly reduce mortality.

PI Name: David Rimm

Mechanism: Investigator Initiated Research

Institution: Yale University

Application Title: In Situ Detection of Cancer Stem Cells to Predict Non-Nodal Metastasis in Breast Cancer

Abstract:

The primary cause of death from breast cancer is metastasis, when cells from the tumor in the breast move through the circulation to other sites in the body and start new tumors at those sites. There are two primary mechanisms of metastasis in breast cancer, through the blood, and through the lymphatics (small channels that lead to the axillary lymph glands) When tumors go to the lymph glands (nodes) that suggests a worse prognosis, but many tumors can still be cured even if they have already achieved that stage. Metastasis to lymph nodes can be detected by lymph node biopsy, and the finding of breast cancer cells in the lymph node dramatically affects choice of therapy, leading to more aggressive therapies. However, there is no current method for finding non-nodal metastasis. These tumors are often only detected when they are symptomatic at a distant site (for example bone pain), when it is too late to successfully treat. Thus there is a significant and immediate clinical need for early detection methods for non-nodal metastasis. Since if these tumors were treated more aggressively at an early stage, then patients might be cured.

This proposal hypothesizes that a new class of cells, recently describe in breast cancer may be the key to early detection of non-nodal metastasis. These cells are called either cancer initiating cells or cancer stem cells (CSCs). One prominent theory in the stem cell field is that these cells are the key cells that maintain a cancer. These cells are thought to divide slowly and thus are not susceptible to some less aggressive cancer therapies. We believe that the number of CSCs per tumor will reflect the aggressiveness of the tumor. However, these CSCs are morphologically indistinguishable from other tumors cells, and thus cannot easily be identified or counted. Most of the proteins made by these cells are similar to those made by many other cancer cells. However, there are thought to be a few distinguishing molecular features that can uncover these cells. We and others have proven that some of these proteins are associated with CSC properties and also bad outcomes in breast cancer. Here we propose to use the first year or two perfecting a microscopy-based assay to definitively identify these cells in breast cancer pathology sections. In the meantime, we will be collecting specimens from our archives from patients that historically had low stage tumors, but ultimately suffered from non-nodal metastasis (while we are also collected a control set that is similar, but without the metastasis). Finally, in year 3, we will test our new assay for counting CSCs on this case-control cohort to determine if our hypothesis is correct. That is, does counting CSCs reveal a threshold number, above which tumors have a high likelihood of non-nodal metastasis.

Our vision for this test would be as a diagnostic test for breast cancer that is complementary to Oncotype Dx. Oncotype Dx selects patients with aggressive cancer based on recurrence scores, but it does not use any markers based on CSCs. We anticipate that our test would be independent from the Oncotype Dx test, and help triage the 60-80% of patients who are not definitively characterized by that test. Finally, the methods we are using, based on the

Pending Execution of Grant Agreements

AQUA microscopy platform could be readily commercialized if successful. They are based on formalin fixed, paraffin embedded

PI Name: Steven Isakoff

Mechanism: Investigator Initiated Research

Institution: Massachusetts General Hospital

Application Title: Functional Metabolic Near-infrared Tomographic Optical Breast Imaging (TOBI) to Monitor Response to Neoadjuvant Therapy in Breast Cancer

Abstract:

Public Abstract Functional Metabolic Near-infrared Tomographic Optical Breast Imaging (TOBI) to Monitor Response to Neoadjuvant Therapy in Breast Cancer

Breast cancer is the most common cancer in women in the United States with over 182,000 new cases estimated in 2008. A major goal in the treatment of breast cancer is to individualize therapy to increase the likelihood that a patient will receive the most effective therapy while minimizing the risks of using an ineffective treatment. To reach this goal we need to develop better tools to predict both the risk of relapse of an individual tumor and the likelihood that the tumor will respond to a specific therapy. We also need better tools to determine early in the course of treatment whether a patient is responding to therapy so that ineffective therapy can be changed to more effective therapy. The overall goal of this project is to develop and test a new noninvasive, inexpensive imaging technology, called functional optical breast imaging, to monitor the response to therapy. This technology can detect changes in the physiology of breast tumors that may predict whether a tumor is responding to therapy soon after the initial treatment.

Several large clinical trials have shown that therapy given before or after surgery results in similar success in preventing tumor recurrences. However, preoperative therapy allows more patients with locally advanced breast cancer to have breast conserving surgery. Patients treated preoperatively who had no evidence of cancer at the time of surgery had two-thirds fewer recurrences compared to patients who had residual cancer after preoperative treatment.

Currently, methods to monitor response to preoperative therapy and predict response early in treatment are either too expensive or have inadequate sensitivity and specificity. Common techniques such as ultrasound, mammography, or magnetic resonance imaging (MRI) generally correlate poorly with findings at surgery and do not show changes early enough to permit changes in treatment selection. Positron emission tomography (PET) scans have been shown to predict a final response after 1 or 2 cycles of therapy, but are expensive, invasive, and have limited availability. Therefore, improved strategies are needed to assess the effectiveness of preoperative therapy as early as possible in the treatment of breast cancer to minimize side effects when tumors are not responding and provide the opportunity to switch to another potentially more active agent.

This proposal aims to evaluate a new advanced optical imaging system to predict response to preoperative therapy in breast cancer. In particular, we will test the hypothesis that changes seen early in the course of treatment using our optical imaging device will predict the response to treatment at the time of surgery. This technology is noninvasive, inexpensive, portable, and will provide physiological information about the tumor not available by current methods. Because the technology is noninvasive and uses near-infrared light, similar to a strong flashlight, there is virtually no risk to the patient. This technology will be able to determine the oxygen consumption and blood flow within the tumor, the water

Pending Execution of Grant Agreements

concentration, fat concentration, and oxy and deoxy-hemoglobin concentrations in the tumor (which reflect how efficiently blood is supplied to the tumor). This information will be analyzed to correlate physiologic changes to treatment response. The first specific aim of this project is to complete the construction of the functional optical imaging device and the data processing tools to analyze the optical data that will be generated. The device will be built within the first few months of the project. The second specific aim of this project will be to conduct a pilot clinical trial to test the functional optical imaging device in a group of 24 patients over 3 years undergoing preoperative therapy for breast cancer at the Massachusetts General Hospital Cancer Center. Eligible subjects will have an initial optical imaging scan prior to treatment, a scan 7 days after the first treatment, and then again with each cycle of treatment. Periodic MRI scans will also be obtained. The data from the optical imaging scans will then be correlated with the final pathology at the time of surgery to determine whether functional changes detected by optical imaging correlate with response to treatment.

The results of this project may have important implications for the future treatment of breast cancer. Patients may be able to learn after just 1 week of treatment whether they are likely to experience a significant response to treatment, providing the opportunity to switch to more effective treatment. In addition, this technology may aid in the evaluation of novel targeted therapies in breast cancer by providing a physiological indicator of tumor response. Although initially patients with locally advanced breast cancer may benefit from this technology, potentially patients with earlier stage breast cancer may also benefit by receiving preoperative therapy that can be individualized based on the predicted response to treatment. Finally, the results of this study will inform the design of future clinical trials to further develop this technology to monitor and predict response to preoperative therapy. This project may have a substantial impact on the treatment of breast cancer by allowing for individualized therapy based on the early evaluation of tumor response, ultimately improving the outcome of patients with breast cancer.

PI Name: Kermit Carraway

Mechanism: Investigator Initiated Research

Institution: University of California at Davis, School of Medicine

Application Title: Induction of apoptosis of highly invasive breast cancer cells by novel small molecule inhibitors of intracellular urokinase plasminogen activator

Abstract:

The major challenge in developing novel and more effective treatments for breast cancer is the identification of drugable targets. Ideally, such targets play major roles in the development or malignancy of breast tumors, but play minor roles in other physiological processes. Thus, therapeutic inhibition of the activities of these targets will specifically thwart tumor cells while eliciting minimal patient side effects. It has been known for almost two decades that urokinase plasminogen activator (uPA) and its cell surface receptor uPAR are overexpressed in malignant breast cancer cells. These components act at the surface of tumor cells to promote metastasis and malignancy, and a drug currently in Phase II clinical trials targets surface uPA to suppress its activity. However, while this drug suppresses the proliferation and metastatic potential of tumor cells, it does not kill the cells, raising the possibility that surviving tumor cells could re-seed tumors and result in recurrence following therapy. Recently, we have discovered that an active form of uPA is present inside of malignant cultured breast tumor cells (intracellular presence), but this active form is not present inside of normal cells. Moreover, we have made the unexpected discovery that the survival of malignant breast tumor cells is dependent on the presence of active intracellular uPA. These observations suggest that intracellular uPA is an ideal target for the development of breast cancer therapies; drugs that inhibit intracellular uPA activity are expected to promote the apoptotic death of malignant tumor cells, but are not expected to affect normal cells because they do not contain active intracellular uPA and are not dependent on uPA or their survival.

A secondary function of the FDA-approved diuretic amiloride is to inhibit uPA enzymatic activity, however it accomplishes this with relatively low efficiency. Interestingly, we have found that amiloride can cross the plasma membrane to access the cell interior, and can inhibit intracellular uPA and induce malignant breast tumor cell death at high concentrations. Based on these observations we have developed a series of amiloride derivatives that display greater efficacy toward intracellular uPA. In addition to chemically modifying the drug so that it acts more efficiently toward the uPA protein, we have made modifications that exploit uPA's enzymatic activity to allow these drugs to become trapped inside the cell and build to high concentrations. As noted above, since enzymatically active uPA is only found in malignant tumor cells, this will lead to the apoptotic death specifically of tumor cells relative to normal cells.

The first objective of the proposed studies is to begin to understand at a molecular level how malignant tumor cells are dependent on intracellular uPA for their survival. We propose that intracellular uPA activity pulls the balance between survival signaling and apoptotic signaling toward survival by engaging biochemical pathways that inhibit cell death. We will test this hypothesis by using our compounds to interfere with uPA activity inside breast tumor cells and assessing the impact on the biochemical pathways characteristic of cellular survival and death.

Pending Execution of Grant Agreements

Our second aim is to begin to assess the translational potential of our amiloride-based intracellular uPA inhibitors. Here we will determine whether our drugs affect the initiation, growth and metastasis of mammary tumors in a transgenic mouse model of breast cancer. A very notable corollary of our studies concerns the potential role of intracellular uPA activity in tumor recurrence following initial treatment. Recurrence of breast tumors in patients is mediated by disseminated tumor cells. These are cells that have detached from the primary tumor (often found in circulation), have evaded killing by conventional therapies, and go on to re-seed the tumor often at a distal or metastatic site. Expression of uPA and uPAR has been observed in these cells, raising the possibility that intracellular uPA actively contributes to the survival of this particularly therapy-resistant tumor cell population. We will test this hypothesis using a transgenic mouse model where the tumor-driving oncogene can be turned on and off in the mammary gland. This cycle results in the formation and regression of tumors, but in this model tumors spontaneously recur after a characteristic latency even though the driving oncogene remains dormant. We will test the role of intracellular uPA in mammary tumor recurrence by determining whether our inhibitors interfere with this spontaneous recurrence. If so, such observations would point to the therapeutic benefit of treating patients with intracellular uPA-directed therapies following conventional therapies.

PI Name: Pepper Schedin

Mechanism: Investigator Initiated Research

Institution: University of Colorado Health Sciences Center

Application Title: Targeting the pro-inflammatory milieu of the involuting gland to suppress pregnancy-associated breast cancer metastasis

Abstract:

Public Abstract Young women diagnosed with breast cancer are more likely to die from their disease than older women. In fact, young age at diagnosis is a predictor of poor outcome that is independent of other known negative breast cancer specific factors such as size of tumor or lymph node involvement. Why young women's breast cancer has a poor prognosis remains unknown. While most people are aware that having a pregnancy at an early age reduces one's risk of developing breast cancer over a lifetime, most are unaware that the cancer protective benefit of an early-age pregnancy does not affect breast cancer risk until decades later. Further, women who delay child bearing to their thirties are actually at increased risk of developing breast cancer over their lifetime in comparison to women who have never been pregnant. Importantly, for all recently pregnant women, the risk of breast cancer actually increases for up to ten years before falling to protective levels. In addition, recent data has shown that women diagnosed with breast cancer shortly after pregnancy are almost twice as likely to die from their disease. Breast cancers diagnosed around pregnancy have been called pregnancy-associated breast cancer (PABC) and clinicians are only just beginning to realize that young patients with a recent pregnancy are at very high risk of disease progression.

The prevailing explanation for why pregnancy-associated breast cancer has such a poor prognosis is that the hormones of pregnancy promote breast cancer. Newer data suggest that in fact, events following pregnancy, during the time when the breast tissue returns to its pre-pregnant state, are more important in promoting metastasis than events that occur during pregnancy. The regression of breast tissue after pregnancy to its pre-pregnant state is called involution. In this grant, we will test the hypothesis that pregnancy-associated breast cancer has a poorer prognosis because breast involution actively promotes metastasis. Our lab has shown that as breast tissue regresses after pregnancy, the gland becomes pro-inflammatory, that is, the breast uses wound healing programs to help return the gland to its pre-pregnant state. We have shown that this occurs in mouse and rat models and more recently in breast tissue biopsied from women who were pregnant, lactating, or whose breasts were undergoing weaning-induced involution at the time of the biopsy. Specifically, in the involuting gland, we observe increased immune cell influx, turnover and breakdown of the layer that typically surrounds the normal breast duct (stroma), as well as evidence of increased enzymes that help breakdown these layers and which may permit escape of tumor cells from the breast. While this inflammation is normal, we propose that it enhances tumor cell growth and metastasis, and accounts for the aggressiveness of breast cancer in young women who have recently been pregnant. In preclinical animal models, we have shown that the pro-inflammatory changes that occur in the involuting breast are capable of promoting breast cancer metastasis. These data support our hypothesis that reducing the pro-inflammatory nature of the involuting breast will reduce the incidence and aggressiveness of PABC.

Pending Execution of Grant Agreements

In this project we will determine whether treating animals with the anti-inflammatory agent fish oil or ibuprofen during involution reduces the pro-inflammatory nature of the involuting gland. We will determine whether such treatments suppress growth and metastasis of mammary tumors that are promoted by involution. We propose to use three independent animal models, each having its own strengths and limitations. If data from all three models demonstrate that the pro-tumorigenic attributes of involution can be suppressed by the use of these anti-inflammatory agents, we will be in a position to test this theory in women. It has been argued that the tumor promotional contribution of mammary involution is a low level risk factor (Relative Risk ranges from ~1.1-1.3). However, it is an exceptionally high expressivity risk factor, affecting a large proportion of the female population. Recent data from the National Vital Statistics System recorded over 3.5 million births in the US in 2004. These women represent a readily identifiable, high risk population that may benefit from prevention treatment. If the conservative upper limit of 35 years of age for reproductive potential is chosen, an estimated 25,000-30,000 breast cancer patients per year in the US may have a recent pregnancy as a negative prognostic feature. If successful, the pre-clinical results obtained from these proposed studies could translate to the clinic within 10 years. The 'clinic' would be the obstetrician's office and the woman's treatment would be a preventive agent (postnatal pill) taken during the window of time when her breast tissue is in the 'drying out' period that follows delivery (if she does not nurse) or weaning (if she does). In summary, we have identified a unique period of mammary gland biology, i.e., involution, as a potentially important target for breast cancer prevention. By targeting prevention strategies during breast involution, the length of treatment will be defined and of relatively short duration, as involution in women is largely completed within 6 months of initiation. In addition, such limited treatment exposure is anticipated to reduce treatment side effects. The goals of limiting treatment duration and reducing side effects are critical objectives in cancer prevention trials, and as such, these strategies are anticipated to be well received by physicians and high risk patients alike.

PI Name: Heather Eliassen

Mechanism: Investigator Initiated Research

Institution: Brigham and Women's Hospital

Application Title: Plasma marker of oxidative stress and risk of breast cancer: a prospective study

Abstract:

This study proposes to investigate the association between a plasma marker of oxidative stress and breast cancer risk. Oxidative stress may play an important role in the development of breast cancer and metastasis through its ability to damage lipids, protein, and DNA. Fluorescent oxidation products, measured in the blood, are an innovative marker of overall oxidative stress and have been associated with an increased risk of cardiovascular disease, but to our knowledge, have not been investigated in relation to breast cancer risk. Although generated as part of normal processes, reactive oxygen species (ROS) may be destructive within cells, causing damage to lipids, proteins, and DNA that could lead to cancer. Under normal circumstances ROS generation is carefully balanced by destruction of ROS by the body's enzymes and clearance of ROS by antioxidants taken in from the diet. Transient fluctuations in ROS levels are part of normal cellular regulation, but if ROS generation overwhelms the antioxidant defense system (e.g., by exposure to external sources of ROS and insufficient antioxidant intake or enzymatic clearance) a state of oxidative stress can result.

While oxidative stress-induced DNA damage may relate to several types of cancer, there is specific evidence to suggest it plays a particular role in the development of breast cancer. First, several established breast cancer risk factors are potential contributors to ROS, including estrogen, alcohol, and ionizing radiation, suggesting that generation of ROS may be at least one of the mechanisms through which these factors influence disease risk. Second, plasma carotenoids have been associated with reduced breast cancer risk, suggesting that their antioxidant activity may counter oxidative stress. Finally, levels of oxidative stress markers have been found to be higher in breast cancer tissue compared with normal tissue, suggesting that oxidative stress is directly involved in the origin of breast cancer.

Several small studies of breast cancer have evaluated two lipid-specific plasma markers with consistent results finding higher levels of these markers in cases than in controls. However, these retrospective studies assessed the plasma markers after breast cancer diagnosis in the cases. While these results are intriguing, retrospective studies of oxidative stress markers are difficult to interpret given that higher levels in cases may be caused by the tumor or by treatment of the disease rather than oxidative stress being a cause of disease. In addition, the focus on lipid-specific biomarkers may have ignored other potentially important oxidative products. A novel assay measuring fluorescent oxidation products represents a more global measure of oxidative stress encompassing oxidation from many sources including lipids, proteins, and DNA. Plasma fluorescent oxidation products have been associated with several oxidative-stress related exposures and with the risk of coronary heart disease. Thus, these studies provide support that fluorescent oxidation products appear to be physiologically relevant and representative of exposure to oxidative

Pending Execution of Grant Agreements

stress. To our knowledge, no prospective studies of the association between biomarkers of oxidative stress and breast cancer risk have been conducted.

We are proposing to assess the association between the global fluorescent oxidation products marker and breast cancer risk in the prospective Nurses' Health Study (NHS). In the NHS, blood samples were collected in 1989-1990 from 32,826 women and have been frozen since collection (stored at -130°C). We will use a nested case-control design and measure the oxidative stress assay among 775 breast cancer cases (and their matched controls) diagnosed 1992-1998. This study provides an ideal context in which to investigate these associations, given the prospective nature, extensive covariate information, and an ongoing, high rate of follow-up (98% in the blood cohort).

The proposed study will assess a biomarker of risk and incorporate a novel approach to enhance our understanding of the breast cancer disease process. We also feel this study will make a significant contribution to the goal of reducing the burden of disease over the next decade. Finding a significant association between this marker and breast cancer risk would help shed light on the role of oxidative stress in the development of breast cancer and would introduce a new measure to help identify women at risk of developing breast cancer. The results of this work, along with our understanding of the antioxidant defense system, would lead to the next step to determine the best way to reduce levels of oxidative stress, such as reducing exposure to ROS contributors and increasing exogenous intake of antioxidants (e.g., carotenoids in fruits and vegetables). Thus, our proposed study would be an excellent contribution to expanding our knowledge and reducing the incidence of breast cancer.

PI Name: Andrea Richardson

Mechanism: Investigator Initiated Research

Institution: Dana-Farber Cancer Institute

Application Title: Lysosomal Associated Protein Transmembrane 4B (LAPTM4B), a novel drug resistance gene in breast cancer

Abstract:

The research proposed in this Susan G. Komen grant application comes from Dr. Andrea Richardson, a pathologist, and her colleague Dr. Zhigang Charles Wang, a molecular biologist. This team discovered a genetic alteration in breast cancers that promotes metastasis and may lead to chemotherapy resistance. The application focuses on resistance to chemotherapy and the way in which this happens.

The alteration is an increased number of copies for the gene LAPTM4B, which encodes a protein that resides in a cellular structure called the lysosome. When this gene is amplified, and too many copies are present in the tumor, we believe cancer cells become refractory to doxorubicin (marketed as Adriamycin), the most common chemotherapy drug used in breast cancer treatment. Our application will investigate this discovery more fully. The LAPTM4B protein is located on lysosomes, and lysosomes are intracellular vessels that contain an acidic environment and carry destructive enzymes. Lysosomes are responsible for destroying proteins and “cleaning up the trash” both inside and on the outside of cells. Adriamycin can end up in lysosomes, and in cells with too much LAPTM4B, we believe more Adriamycin is restrained in lysosomes and is restricted from the nucleus of the cell. To be an effective anti-cancer agent, Adriamycin needs to get to the nucleus. Therefore, a protein that keeps the drug in the lysosome will protect the tumor cell and lead to treatment resistance. Gene amplification leads to protein over-expression, and in this case, too much LAPTM4B. This over-abundance of LAPTM4B leads to retention of Adriamycin in lysosomes, and perhaps interferes with the normal function of lysosomes. The end result is cancer cells that resist destruction and are more impervious to chemotherapy. This is our working hypothesis, and will be explored, tested and applied in our research. By using breast cells with high levels of LAPTM4B, or by genetically manipulating the levels of the protein, we will determine why cells are resistant to Adriamycin and if they are resistant because the drug can't get to the nucleus of the cell and can't damage the DNA of the tumor. We will examine the function of the lysosome in cells with high and low LAPTM4B levels in order to see whether altered function can explain treatment resistance. We will mimic the situation in human tumors by using breast cancer cells that grow tumors in animals. By manipulating the level of LAPTM4B, a direct test of treatment resistance to chemotherapy will be performed in these “pre-clinical” animal models. Finally, the effect of increased copies of the LAPTM4B gene in human cancer will be examined in women receiving Adriamycin after curative surgery for their breast cancer (adjuvant therapy). We will assemble a large cohort of women treated at the Dana-Farber Cancer Institute and Brigham and Women's Hospital in Boston for whom we have follow-up information and who received chemotherapy based on Adriamycin. Additional confirmation will be sought in cohorts of patients treated in national, multi-institutional clinical trials.

This research can immediately provide a potentially useful biomarker for predicting response to the most widely used chemotherapy for breast cancer. The biomarker,

Pending Execution of Grant Agreements

LAPTM4B gene copy number or protein over-expression, is a “de novo” biomarker for resistance, in other words, it is present prior to treatment and predicts whether or not a tumor will respond to a drug before the drug is given. This is a great advantage to doctors, who might be able to alter the drugs prescribed based upon the biomarker test. The research may also provide a viable target for improving therapy in the future. If high LAPTM4B levels lead to chemotherapy resistance, or unnatural viability of cancer cells, then its reduction should be therapeutic. There are strategies to reduce LAPTM4B levels, and drugs targeting lysosome function are under development. In the future, knocking out LAPTM4B function may extend the usefulness and increase the therapeutic window of breast cancer chemotherapy.

PI Name: Julie Palmer

Mechanism: Investigator Initiated Research

Institution: Trustees of Boston University, BUMC

Application Title: Genetic variants and gene-environment interactions in relation to breast cancer incidence in African-American women

Abstract:

Breast cancer is the most common cancer in African American women, and death rates from breast cancer are higher among African-American women than other American women. If a woman has a so-called “breast cancer gene”, such as BRCA1, she has a very high risk of developing breast cancer during her lifetime. However, most women who develop breast cancer do not have a high risk gene like BRCA1. Rather, it is likely that many common genes together, each with a very small effect, contribute to a woman’s risk of breast cancer, and that these genes with small effects account for the vast majority of breast cancer occurrence. We hypothesize that there are some common genetic variations that are present more often in African American women with breast cancer than in African American women without breast cancer. Identifying these variants will lead to a better understanding of the mechanisms of breast cancer development and thus to better treatments for breast cancer. In addition, the information on genetic variants can be used to identify subgroups of women at greatest risk of the disease.

The study hypothesis can be tested by examining DNA from 1200 African American women with breast cancer and 2400 African American women without breast cancer, all of whom are participants in the Black Women’s Health Study (BWHS) of Boston University. In recent years, techniques have been developed that allow researchers to examine hundreds of thousands of genes at once in relation to risk of a disease. Three studies of this type, called genome-wide association studies, have been carried out in European ancestry (i.e., White) populations and have identified a few genetic variants that appear to contribute to breast cancer risk in Whites. A similar study is underway among Asian women from Shanghai. In our study, we propose to determine whether the genetic variants identified in White women and Asian women also cause breast cancer in African American women. In addition, we will examine whether there are certain groups of African American women with the particular genetic variant who are at particularly increased risk. For example, it could be that obese women are more affected than women who are not obese. Combining information from genes and lifestyle factors will help to identify African American women who are at very high risk of breast cancer.

To determine whether the genetic variants identified in genome-wide association studies of White or Asian women are associated with breast cancer risk among African American women, we will use DNA samples and questionnaire data collected in the BWHS, a follow-up study of the health of 59,000 African American, in progress since 1995. The BWHS has a repository of saliva samples provided by 26,700 BWHS participants, from which DNA is extracted for use in specific genetic studies. Through follow-up health questionnaires every two years, the BWHS has also collected a wealth of information on potential risk factors for breast cancer, including reproductive history (age at start of menstruation, age at first birth, age at menopause, type of menopause), family history of breast cancer, female hormone use, participation in exercise, body mass index, and dietary intake. We propose to carry out

Pending Execution of Grant Agreements

genetic assays of DNA from 1200 women with breast cancer and 2400 women without breast cancer to assess which, if any, of the genetic variants that have emerged from genetic studies of White or Asian women are associated with breast cancer risk in African American women. For the variants that we find to be associated with risk in the BWHS, we will study whether the effect is greater in women with certain characteristics; that is, we will assess gene-environment interactions. For example, women who have a key genetic variant and are obese or whose menstrual periods began at an early age could be found to be at particularly high risk of breast cancer. In these analyses, we will assess reproductive factors, body mass index, and participation in physical activity, all of which are thought to influence breast cancer risk.

In sum, the proposed study will identify genetic variants that may influence risk of breast cancer in African American women. This information is likely to lead to more effective treatments for breast cancer. The information gained from the proposed study may be especially helpful in developing treatments for estrogen-receptor negative (ER-) breast cancer, which typically has a worse prognosis and does not respond to Tamoxifen or other medications that block endogenous hormones. The proposed study is responsive to the objectives and research focus of the 2008/2009 Investigator-Initiated Research RFA in that it is a molecular/genetic epidemiology study. In addition, the research, by identifying genetic variants associated with breast cancer in African American women, will have significant potential to lead to reductions in disparities in breast cancer incidence within the decade.

PI Name: Deborah Rhodes

Mechanism: Investigator Initiated Research

Institution: Mayo Clinic and Foundation

Application Title: Evaluation of Low-dose Molecular Breast Imaging as a Screening Tool in Women with Mammographically Dense Breasts and Increased Risk of Breast Cancer

Abstract:

About half of the reduction in breast cancer mortality since 1989 can be attributed to the use of screening mammography (SM). However, not all women are equally well served by SM: mammography finds about 6 out of 10 breast cancers in women with dense breast parenchyma, and about 3 out of 10 breast cancers in women with a strong family history of breast cancer. Digital mammography and screening ultrasound are only slightly better than conventional mammography in finding cancers in these women. MRI is much more accurate, but costs ten times more than SM. This high cost may considerably limit access to MRI as a screening test.

We have developed a new technology called Molecular Breast Imaging (MBI) using two gamma cameras to image the breast. Unlike SM, MBI relies on gamma radiation rather than x-rays to detect cancer. One advantage of our MBI system is that the ability to detect breast cancer is not reduced by dense breast tissue. Another advantage is that an MBI scan is painless, using only light pain-free compression of the breast tissue, in contrast to the forty pounds of force applied to the breast during SM. A third advantage is that the MBI system will be far less expensive than an MRI to construct and far easier to interpret, making it much less costly for the patient. In studies to date, we have found that MBI is as accurate as MRI in detecting breast cancer.

With a grant from the Susan G. Komen Foundation, we performed screening MBI in 980 women who had dense breast tissue and were at increased risk of developing breast cancer. In these women, we found a total of 12 cancers. MBI detected 10 of the 12 cancers, SM detected 3 of the 12, and one cancer was not visible on either MBI or SM. Thus, MBI detected 3 times more cancers than SM. The main limitation of this study is that the radiation exposure to the patient from the MBI study was significantly higher than the radiation exposure from SM.

We have determined how to lower the radiation dose of MBI by a factor of five, such that the radiation dose would be just slightly higher than that of SM. With the anticipated implementation of these dose reduction techniques by January / February 2009, we are seeking funds to repeat the MBI screening study at the lower radiation dose.

We hope to demonstrate that MBI can detect 3 times more breast cancers than SM at a radiation dose that is comparable to SM. We plan to invite 1000 women with dense breast tissue and increased risk of breast cancer to undergo low-dose MBI. These women will have both an MBI and SM performed and interpreted independently. Although this is being done as a research study, we will inform all patients with an abnormal MBI of our recommendation for additional diagnostic evaluation, and we will help to coordinate the additional evaluation. Patients with an abnormal MBI but normal further diagnostic evaluation will be invited to return for a six-month follow-up MBI. At fifteen months after enrollment in the study, we will contact patients to determine if they have developed a cancer that might have been missed on MBI and SM.

Pending Execution of Grant Agreements

SM has already led to a substantial reduction in the death rate from breast cancer. Imagine how many more lives could be saved if we had a technology that found three times more cancers with no additional radiation and little additional cost?

PI Name: Rakesh Singh

Mechanism: Investigator Initiated Research

Institution: University of Nebraska at Medical Center, Eppley Cancer Center

Application Title: Development of Molecularly Targeted Therapeutics for Osteolytic Bone Metastasis

Abstract:

Breast cancer is the most common cancer and the second leading cause of cancer-related death in women in the United States. Breast cancer cells show a strong predilection for metastasis to bone and most complications of breast cancer are attributed to bone metastasis. Bone metastases in breast cancer are predominantly osteolytic, leading to pathological fracture, intractable bone pain, nerve compression and hypercalcemia. These complications are not only potentially lethal but also decrease the quality of the life. Current therapies aimed at improving the quality of life, symptom control and prolongation of survival in breast cancer with bone metastasis are not effective, and often associated with severe toxicity. Therefore, understanding the molecular mechanisms underlying the bone metastases is critical to develop novel treatment strategies with no or limited toxicity for effective management of advanced breast cancer patients with metastatic disease. Our long term goal is to improve the therapeutic strategies for osteolytic bone metastasis in breast cancer with a better understanding of the biology of bone metastasis. Our specific objective is to develop novel therapeutic strategy to inhibit osteolytic bone metastasis. A recent report from our laboratory demonstrated that Cathepsin G-dependent cleavage of receptor activator of nuclear factor-kappa B (RANL) ligand (RANKL) to generate soluble RANKL (sRANKL) promoted osteoclast activation in breast cancer. However, the generation and functional significance of sRANKL at the TB-interface in breast cancer bone metastasis remains unclear. Our central hypothesis is that inhibition of sRANKL generation the tumor-bone interface in combination with cytotoxic drug therapy in osteolytic bone microenvironment will abrogate establishment of breast cancer bone metastasis. Our rationale for these studies is that development of novel approaches to modulate expression of molecular targets critical in tumor-bone interaction during osteolytic bone metastasis, and targeted delivery of chemotherapeutic drug to osteolytic microenvironment to inhibit malignant cell proliferation. The studies proposed in this application are highly innovative and will have significant impact on development of novel targeted therapeutics in coming decade. We will target Cathepsin G expression and sRANKL generation, which is up-regulated during tumor bone interaction. Moreover, we have identified these genes using a unique mouse model and will test their functional role in experimental and spontaneous osteolytic bone metastasis in syngeneic settings. Furthermore, targeting functionally defined genes up-regulated at the TB interface will allow us to develop the next generation of molecular diagnostics and therapeutics in the field of breast cancer bone metastasis. In addition, we will develop a novel therapeutic approach using an osteotropic drug delivery system to target genes in the resorptive bone microenvironment, to inhibit osteolytic bone metastasis. In summary, the knowledge gained from these studies will lead to development of innovative approaches to inhibit tumor induced osteolysis and malignant cell proliferation in bone microenvironment using our novel delivery system for development of highly effective therapy for osteolytic bone metastasis with minimal toxicity.

PI Name: David Potter

Mechanism: Investigator Initiated Research

Institution: University of Minnesota at Twin Cities

Application Title: A Phase I/II Trial of Short Course Pre-Operative Ritonavir in Breast Cancer

Abstract:

HYPOTHESIS: Although there have been many recent advances in breast cancer treatment and many treatment options, 40,000 American women still die each year due to breast cancer. Our goal is to make a significant contribution to the struggle to decrease mortality due to breast cancer. Recently, we have demonstrated that the HIV protease inhibitor, ritonavir, which is FDA approved for AIDS, exhibits anticancer activity in a mouse breast cancer model at clinically attainable drug levels. We have identified two key regulatory proteins in breast cancer biology that are inhibited by ritonavir. One protein is called Hsp90. Hsp90 affects multiple pathways in breast cancer growth by folding proteins important for cancer growth and is therefore at the crossroads of breast cancer biology. Ritonavir blocks the function of Hsp90. Ritonavir also blocks the activity of drug metabolizing enzymes called cytochrome P450 enzymes (or CYP's), which were thought to metabolize drugs. Recently, we have recently found that cytochrome P450 enzymes play a role in cancer progression by making specific fatty acid oxidation products that are naturally found at low levels in the body. Hsp90 and the oxidized fatty acid signaling molecules both promote activation of a signaling protein called Akt kinase, which in turn promotes cancer cell survival. We propose to test the hypothesis that ritonavir inhibits the growth of breast cancer by blocking Hsp90 and cytochrome P450 function, thereby depriving Akt kinase of the tools it needs to promote breast cancer growth. This hypothesis will be tested in a clinical trial of novel design.

HOW THE STUDIES UNIQUELY ADVANCE OUR UNDERSTANDING OF BREAST CANCER: To re-purpose ritonavir for breast cancer therapeutics, we recognize that we cannot tell from preclinical data which group of breast cancer patients is likely to benefit from ritonavir. A recent example of this is the finding that the drug erlotinib was more effective in ERa+ breast cancer than in triple negative, contrary to expectations from pre-clinical studies. We therefore propose an open design trial where we will test the activity of ritonavir in ERa+, HER2+ and triple negative breast cancer. This proposal is directed toward funding for the triple negative arm of the trial. To speed development, we propose using a "window of opportunity" design in which we administer this oral drug to patients. Women about to undergo lumpectomy or mastectomy would be treated for a period of 5 or 10 days, the length of treatment determined by an animal model. The diagnostic biopsy performed before the patient enters the study is compared to the removed tumor for signs of response to ritonavir. No additional procedures are required other than two blood draws. The threshold for toxicity is low, so any grade 2 toxicity will result in patients coming off the trial. Most of the toxicity is expected to be gastrointestinal. A phase I component of the study will find an acceptable maximum tolerated dose (MTD). Patients will then be treated with this dose. We need to accrue 24 patients for each arm of the study to determine whether ritonavir is active that arm and there will be three arms as described above. What is novel and paradigm-shifting about this work is the idea that an approved oral drug, ritonavir, may be "re-purposed" for breast cancer through an unbiased and open clinical trial design that is independently powered for triple negative, post-menopausal ERa+, and HER-2+ breast

Pending Execution of Grant Agreements

cancer. Also novel is the use of functional biomarkers in the tumor that are expected to better detect the activity and inhibition of Hsp90 signaling pathways. This proposal seeks support the triple negative arm of the trial.

CLINICAL IMPACT: The results of the studies proposed here can be rapidly translated to phase II clinical trials of ritonavir in recurrent/metastatic breast cancer, yielding a roadmap for clinical application in breast cancer within the next 10 years. The activity of ritonavir in a breast cancer xenograft model argues strongly that it could be re-purposed for breast cancer treatment and its strong inhibitory activity against Akt kinase makes it competitive as a novel treatment for this pathway. The advantage of ritonavir over Akt and PI3-kinase inhibitors now in early phase development, is that ritonavir can be developed faster. Ritonavir has known toxicity, has been already approved for clinical use and inhibits the same pathway. Ritonavir is a novel candidate therapeutic agent now recognized as having significant potential in cancer therapeutics, as evidenced by emerging clinical trials. To date, there has not been an organized effort to develop HIV protease inhibitors for breast cancer. The way to develop ritonavir most efficiently is to utilize open clinical trial design and window of opportunity studies to test biological activity. Until these proposed studies are performed, we won't know which type of breast cancer is best treated with ritonavir. The importance of open design clinical trials cannot be overestimated, because we cannot predict accurately from preclinical studies which populations of patients are likely to benefit from novel therapeutics. The window of opportunity design employed for these studies also provides us with rapid information on the bioavailability and activity of ritonavir. When completed, the proposed studies will tell us how to use ritonavir clinically to help reduce breast cancer mortality, significantly contributing to progress in breast cancer therapeutics within the next decade.

PI Name: Jen-Tsan Chi

Mechanism: Investigator Initiated Research

Institution: Duke University

Application Title: Mammary epithelial cell types as determinant of hypoxia responses in breast cancers

Abstract:

The oxygen is essential for the survival and metabolism of all human cells. When cells experience inadequate amount of oxygen (called hypoxia), these cells make significant changes to adapt to this unfavorable situations. These changes include how cells generate and consume energy; increase their tendency to move and other changes in cell properties. These cellular responses to hypoxia (low oxygen) are called hypoxia responses. These hypoxia response signal very unfavorable and harmful events for breast cancer patients since these changes often lead to more tumor progression, more aggressive invasion, cancer metastasis and resistance to chemotherapies and radiation therapies. Given these known risks associated with hypoxia in tumors, many researchers have tried very hard to target the hypoxia response to decrease their harms and improve patients' outcomes. Since the "hypoxia" responses usually occur when there is not enough oxygen, these hypoxia responses in tumors are often thought to be caused by the low oxygen tension present in the breast cancers. If this is the case, the best way to reduce these harms of hypoxia response is to increase the oxygen tensions in the tumors. Many treatments have been developed to achieve this goal of increasing the oxygen in the tumors. But these treatments do not provide benefits for all treated patients. There are accumulating evidences that our assumption that the hypoxia responses are simply caused by low oxygen level in tumors is incorrect. Therefore, we would need to understand the reasons for strong hypoxia in some breast cancers to identify entirely different ways to mitigate the hypoxia response and improve the clinical outcomes.

At least two distinct cell types are present in the normal breast duct. These two cell types are "luminal" and "basal" cells. Luminal cells line the luminal surface of the duct with secretory properties. On the other hand the basal epithelial cells are present in the basal layer that have properties of both muscle cells and epithelium. Although the target cell(s) for breast carcinogenesis has not been fully elucidated, however it is clear that human breast cancer can be categorized into subtypes that have properties consistent with derivation from both basal and luminal normal cells called basal- and luminal-type breast cancers. This proposal is based on our unexpected findings that almost all "basal" type breast cancers have very strong hypoxia response while "luminal" type breast cancers have relatively low level of hypoxia. The study hypotheses for this proposal are that epithelial cell type (basal vs. luminal epithelial cells) is a major determinant of the degree of hypoxia response in breast cancers because these two cell types have different genetic circuitry responsible for hypoxia responses. Therefore, these cell type-specific differences can be manipulated to reduce the hypoxia responses and improve the clinical outcomes in breast cancers. To test this novel hypothesis, we will first use DNA chips to compare the hypoxia these two types of normal breast epithelial cells. Afterwards, we will also use DNA chips to test whether such a difference in hypoxia response is also present in several breast cancer cell lines reflective of their basal and luminal cell lineage. Finally, we will test the possibility that

Pending Execution of Grant Agreements

we can interrupt this basal cell specific high level of hypoxia regulators to reduce the hypoxia response and sensitize breast cancer cell lines to chemotherapeutics and radiation therapies.

The understanding of these parameters and cellular responses to hypoxia is likely to provide important information on the genetic, metabolic status of individual breast cancers. The experiments proposed here will lead to dramatic changes in how we combat the harmful hypoxia response and improve patients' response to cancer treatments of breast cancers.

PI Name: Shehnaz Hussain

Mechanism: Career Catalyst Research

Institution: University of California at Los Angeles

Application Title: Nucleotide variation in the prolactin receptor and its agonists and breast cancer risk

Abstract:

BACKGROUND: Breast cancer is a familial disease, as evidenced by its tendency to clustering among female relatives in about 20-25% of individuals. However, known genetic predispositions such as BRCA1/BRCA2 mutations, explain less than one quarter of these instances of familial clustering. Thus, there is a critical need to explore the possibility that other inherited factors may explain the risk experienced by these families. This could improve the ability to assess the risk for breast cancer among women in these families and allow earlier detection and possible prevention, with the ultimate goal of reducing mortality from breast cancer. Reproductive factors, such as the number of pregnancies and breast feeding, are consistently shown in epidemiological investigations to be associated with a woman's risk of developing breast cancer, yet the molecular mechanisms for these associations are not completely understood. Prolactin (PRL) is a pituitary hormone that is intimately involved in the production of milk. The prolactin receptor (PRLR), activated in the breast epithelium of pregnant and lactating women, causes remodeling of breast tissue to prepare for lactation. There is accumulating experimental evidence that deregulated PRLR activation predisposes breast tissue to carcinogenic events. PRL is the primary activator of the PRLR, however other related hormones, including growth hormone (GH), placental lactogen (PL), and PRL, can also activate PRLR.

HYPOTHESIS & SPECIFIC AIMS: The hypothesis of this investigation is that polymorphisms (called single nucleotide polymorphisms, or SNPs) in the genes that code for PRLR, PRL, GH, and PL will predispose women with a positive family history of breast cancer to develop breast cancer themselves. The rationale for this hypothesis is that SNPs in these genes may have functional effects on PRLR activation. The specific aims are to identify potentially informative SNPs in these genes and test for an association between these SNPs and breast cancer risk.

METHODS: To address these aims, a case-control study nested in the UCLA Family Cancer Registry will be conducted. This study population, consisting of 494 cases affected with breast cancer and 364 controls not affected with breast cancer, have all donated blood specimens and are well-characterized in terms of genetic, reproductive, and environmental risk factors to breast cancer, and breast cancer diagnostic information. Furthermore, this population is enriched with a positive family history of breast cancer, which increases the likelihood of identifying additional genetic susceptibility factors to breast cancer. SNPs will be selected within a gene to capture the complete pattern of genetic variation across each gene, and those that are selected will also have the greatest potential to affect the function of the gene, as determined from bioinformatic analyses. DNA isolated from donated blood specimens will be used to individually determine genotypes for cases and controls for all selected SNPs, using the SNPlex® and TaqMan® genotyping platforms (Applied Biosystems). Statistical models will be used to test for group differences in genotype distributions between cases and controls, while accounting for the effects of other known or

Pending Execution of Grant Agreements

suspected breast cancer risk factors such BRCA1/BRCA2 mutation status, reproductive factors, and environmental exposure.

SIGNIFICANCE: This study has the potential to uncover genetic factors contributing to the development of breast cancer in women with strong family histories of breast cancer. This type of genetic information is increasingly establishing a role in the clinical setting, and this is expected to grow over the next decade as we learn more about genetic influences on breast cancer development. Currently, it is difficult for clinicians and patients to choose between various management strategies for patients at high risk of breast cancer, ranging from close monitoring to prophylactic surgery, which are associated with various levels of psychosocial and health risks. The goal of this proposal is to identify additional genetic susceptibilities that are either important co-factors to BRCA1/BRCA2, or are important independent breast cancer susceptibility factors among women with a family history of breast cancer who are not BRCA1/BRCA2 mutation carriers. Ultimately, such information will help clinicians individualize screening regimens and other breast cancer prevention strategies for their patients, benefiting patients and their families.

PI Name: Rebecca Riggins

Mechanism: Career Catalyst Research

Institution: Georgetown University

Application Title: Regulation of ERRgamma in endocrine resistant breast cancer by the ERK pathway

Abstract:

Breast cancer is not a single disease. There are several different types that can be separated by their physical and genetic features. Invasive ductal breast carcinomas have historically been the most common type of breast cancer. However, the frequency of invasive lobular breast carcinoma (ILC) diagnosis has been increasing significantly in Western Europe and the U.S. over the past 10-15 years. ILCs most commonly express estrogen receptor alpha (ER), which in many cases is a marker for good prognosis and a sign that these patients are excellent candidates for treatment with antiestrogens like Tamoxifen, or an aromatase inhibitor. Surprisingly, women with ER-positive ILC do not always experience significantly better survival than women with ER-positive ductal tumors when both groups are treated with antiestrogens.

The reasons for this discrepancy are almost entirely unknown, and unfortunately the current laboratory models that are used to study antiestrogen resistance have all been derived from ductal carcinomas. To address this deficiency, we have developed a breast cancer cell culture model of invasive lobular carcinoma that has become resistant to the antiestrogen Tamoxifen. In this model we have found that increased expression of the gene estrogen related receptor gamma (ERRgamma) plays an essential role in Tamoxifen resistance, and that its function is likely to be modified or changed by the activity of a second gene, extracellular signal-regulated kinase (ERK). When we examined ERRgamma in breast tumors, we observed that levels of the ERRgamma gene are significantly higher in the tumors of breast cancer patients who relapsed following Tamoxifen therapy as compared to those women who did not experience recurrence. Importantly, in this study the association of ERRgamma with Tamoxifen resistance was not restricted to women with ILC, which suggests to us that ERRgamma may be a biomarker for poor response to Tamoxifen in many different types of breast cancer. The idea we will be testing is that ERK regulates ERRgamma's function in TAM-resistant breast cancer. In Specific Aim 1, we will use our new cell culture model of invasive lobular carcinoma to understand how ERK regulates or modifies ERRgamma function and whether this explains how these breast cancer cells have become resistant to Tamoxifen. In Specific Aim 2 we will look more closely at ERRgamma expression in breast tumor specimens from 150 patients with ductal and lobular breast cancer in order to determine whether high expression of this gene is linked to poor overall survival, poor response to Tamoxifen, and the activity of ERK. Our innovative studies will advance our understanding of how and why ERRgamma regulates resistance to antiestrogens in breast cancer. These are important clinical problems, and furthering our knowledge in these areas will make a significant impact on breast cancer research as a whole. In the near-term, we anticipate that our findings will specifically benefit the ~128,000 women who develop ER+ breast cancer each year by identifying ERRgamma as a new biomarker for poor response to Tamoxifen. This would allow clinicians and patients to choose a more appropriate therapeutic regimen that will improve overall survival and

Pending Execution of Grant Agreements

disease outcome. Given the recent successes of ERK pathway-specific inhibitors in phase I and II clinical studies we believe that co-measurement of ERRgamma and active ERK in breast tumors might also serve as a rationale for targeted, combination therapy of endocrine agents and ERK inhibitors. Finally, our results will also impact the long-term future of breast cancer research and treatment by helping clinicians and researchers to better understand the molecular biology of the ILC subtype, which is increasingly diagnosed yet poorly understood.

PI Name: Josh Lauring

Mechanism: Career Catalyst Research

Institution: Johns Hopkins University, Kimmel Cancer Center

Application Title: Physiologic modeling of common PI3-kinase pathway mutations in human breast epithelial cells to develop mutant-specific targeted therapies

Abstract:

Although we have made great strides in the early detection and treatment of breast cancer, we are still unable to cure recurrent and metastatic disease with our current therapies. We must constantly work to identify new targets for therapy to further improve outcomes for patients. In recent decades it has been well established that cancers arise after normal cells in the body acquire alterations in the genetic material that instructs the cells how to behave. The search for such cancer-specific genetic changes has led to some of our most successful targeted therapies for cancer, including Herceptin for breast cancer. Only five years ago, researchers discovered that one fourth of all breast cancers carried a mutation in a gene called PIK3CA, which encodes a critical protein that regulates major pathways of cell growth and death. An additional 15-20% of breast cancers carry mutations in other genes in this same growth pathway, known as the PI3-kinase pathway. All of these mutations appear to make the growth pathway hyperactive, as if it were always turned on, even when it should not be. The fact that such a high proportion of breast cancers carry these common genetic changes suggests that PI3-kinase pathway hyperactivation may be essential for most breast cancers to develop. Much has been learned about the role of the PI3-kinase pathway through traditional research methods, but we have used a powerful genetic technique called gene targeting to more accurately model the effects of cancer causing mutations in human breast epithelial cells—the cells that give rise to breast cancer. Using this technique to study the effects of PIK3CA and other PI3-kinase pathway mutations, we have uncovered surprising biological effects that were not revealed by traditional methods. We have discovered that one of the canonical downstream signaling targets of PIK3CA, a protein called mTOR, is not activated by mutant PIK3CA by itself, as had been thought. These findings have profound implications for the design and testing of drugs targeting this pathway in breast cancer, as drugs targeting mTOR are already in clinical trials. In addition we have discovered that mutant PIK3CA activates a distinct signaling pathway that causes tumor growth, revealing a potential novel target for tumors carrying these mutations. Our preliminary data suggests that the drug lithium, which is already used to treat mood disorders, selectively blocks growth of breast cancer cells with PIK3CA mutations. Our hypothesis is that accurate human breast cell models of PI3-kinase pathway mutations common in breast cancer will reveal additional aspects of tumor biology that will improve our treatments. Using paired normal and cancerous human breast cell lines differing only in the presence or absence of a PI3-kinase pathway mutation, we will describe the how the mutations affect cancer cell growth, resistance to chemotherapy and hormonal therapy, and tumor formation. We will determine whether these common mutations can serve as markers to predict sensitivity to hormonal therapies and other targeted therapies. We will identify gene expression “signatures” that distinguish mutant cancer cells from cells with a normal PI3-kinase pathway and use these signatures to find matches in a database of drug-induced gene expression signatures. In addition we will screen a library of over 2000 FDA-approved

Pending Execution of Grant Agreements

drugs for compounds that selectively target tumor growth induced by these mutations, but not normal cells, to identify potential leads for clinical trials or further preclinical drug development. Drugs currently under development are not selective for mutant PI3-kinase proteins and may have excessive toxicities to normal cells in the patient. Our hope is that our research will reveal mutation-specific drugs that have less potential toxicity to normal tissues. Given the fact that PI3-kinase pathway mutations occur in 30-50% of all breast cancers, the ability to target this pathway has the potential to save even more lives than Herceptin and dramatically improve outcomes for breast cancer patients.

PI Name: V Krishnan Ramanujan

Mechanism: Career Catalyst Research

Institution: Cedars Sinai Medical Center

Application Title: Preclinical Fluorescence Lifetime Imaging System For Early Detection of Breast Tumors In vivo

Abstract:

Our perception of perfect health implies that every organ and the cells that make up these organs are working in concert with each other. In this dynamic network of events, any disease can be understood as a mis-step in the global balance that defines perfect health. CANCER IS A DISEASE OF "MISREGULATED CELL GROWTH". Radiological imaging and other clinical technologies have revolutionized cancer diagnosis in recent years. Furthermore, great progress has been made in treating most types of invasive cancers (advanced stage) and these various treatment options have increased the prognosis of cancer survivors. As a way of illustrating this point, according to the recently published "Breast Cancer Facts and Figures 2007-2008" by the American cancer society, the tumor incidence rate has shown two notable trends: (i) From 1988-2000, the incidence rate of small tumors (< 2cm) increased by ~2% per year suggesting the role of mammography and other strategies in detecting early cancers; (ii) From 2000, the incidence rate has continuously decreased by ~3.8% per year revealing the combined success of mammography screening procedures and the awareness for healthy living among women. Despite this good news in advancement in cancer diagnosis, the estimated deaths by 2007/2008 due to breast cancers in the United States are 40,460 !

The FUNDAMENTAL REASON for this paradox is two-fold : (i) Mammography and other clinical radiological procedures have their limitations in achieving spatial resolution below better than a few hundred microns. This limitation is critical because most in situ cancerous lesions are less than this size; (ii) Inability to correlate pre-operative images with the precise location of the tumor during surgery which leads to incomplete resection. This leads to increased tumor recurrence and/or development of more invasive cancers. It is evident that we need alternate diagnosis technologies that can augment the existing repertoire of clinical diagnosis modalities so that the aforementioned fundamental limitations are overcome. This research proposal is a timely attempt to develop a novel, sensitive imaging technology for detecting breast cancer lesions at a very early stage. This proposal stems from the realization that currently available clinical imaging modalities such as mammography screening have their limitations in detecting cancer lesions lesser than a few hundred micrometers in size. Optical imaging in general can provide supreme spatial resolution (~ 0.2 microns) and under right conditions, it is possible to detect even a small group of cancer cells. Fluorescence lifetime imaging microscopy (FLIM) is an emerging technology that overcomes a number of commonly encountered problems in intensity-based optical imaging. With our experience from a variety of FLIM applications and also from pilot experiments in small animal models, we strongly believe that the proposed preclinical FLIM system can diagnose the precancerous lesions at very early stages. More detailed information is given in the Research Proposal section.

HOW DOES THE PROPOSED PROJECT CONTRIBUTE TO SIGNIFICANT ADVANCEMENT IN CANCER DIAGNOSIS ? Surgeons all around the world still employ white light to visualize

surgical areas and to perform surgical procedures. What really determines the successful outcome of any surgery is a optimal combination of the following critical factors, namely,(a) degree of invasiveness of the disease, (b) surgical expertise and most importantly, (c) availability of tangible, quantitative, surgical parameters that can be manipulated by the surgeons and the advanced technologies- to the patient's advantage. One of the major issues in surgical resection of breast tumors is the precise identification of the tumor margin. Radiological procedures including NeoProbe and CT can certainly guide the localization of the tumor-specific contrast agents. There is no currently available real-time quantitative assessment of tumor burden and even those in current vogue do not offer single cell resolution (which is necessary to remove the last fragment of the tumor for successful surgical outcome). Optical imaging is a well-established technology for disease diagnosis. Microscopy techniques that can offer single cell resolution have been in place for more than a few decades. However, the clinical utilization of sensitive, fluorescence imaging modalities is still in its infancy. This proposal aims to fill this gap in knowledge by systematic study of small animal models (preclinical model). The specific aims and task list proposed in this project are founded upon the body of knowledge gained by the Principal Investigator for many years and the confidence that stems directly from the feasibility of the project. The knowledge gained from this preclinical proposal will be very valuable in designing a complete clinical imaging system. At the successful completion of this project, we will have made a complete preclinical fluorescence lifetime imaging modality (for the first time in the history of breast cancer diagnosis) that is geared towards the translational platform for clinical diagnosis of breast lesions in human patients. Although, the proposed system can be a stand-alone diagnostic modality, we believe this system can significantly improve the precision and the accuracy of tumor localization when employed in conjunction with the currently available radiological diagnostic modalities too. Since the principal investigator is teaming with a highly reputed surgical oncologist, the feasibility of making a realistic clinical system is very high.

PI Name: Tari King

Mechanism: Career Catalyst Research

Institution: Memorial Sloan-Kettering Cancer Center

Application Title: An Integrated Genomic Analysis of the Invasive Breast Cancer Risk Associated with Lobular Carcinoma in Situ

Abstract:

Lobular carcinoma in situ (LCIS) is most often an incidental finding in a breast biopsy performed for another reason, yet once a woman is diagnosed with LCIS she faces a much higher risk for the subsequent development of invasive breast cancer than the average woman. Historical data suggests that the lifetime risk of breast cancer is 20-25% and is equal in both breasts, even when LCIS is present in only one breast. As such, patients are offered one of three treatment options: (1) lifelong surveillance with the goal of detecting a subsequent breast cancer at an early stage, (2) tamoxifen chemoprevention, or (3) bilateral prophylactic mastectomy. New research however suggests that all LCIS may not behave in the same way and therefore all LCIS may not confer the same increased risk of breast cancer. The available data are very limited and there is currently no method to identify which LCIS carries the highest risk. The objective of this proposal is to identify different types of LCIS by examining which genes are turned on and off in different LCIS specimens. Our hypothesis is that in some LCIS specimens we will find that the same genes are turned on and are present in invasive lobular breast cancer indicating that women with these particular LCIS subtypes will be the ones that carry the highest risk for the development of invasive cancer. We predict that in other LCIS subtypes a different set of genes will be turned on and hence these particular LCIS lesions will place women at much lower risk for developing invasive breast cancer. The gene sets that separate LCIS into these high-risk and low-risk subtypes will be called gene signatures. Our laboratory is using a combination of the latest molecular technologies to look for these gene signatures. The work described in this proposal builds on our previous work and will identify genes that are thought to be important regulators of other genes. We will look for these regulatory gene signatures by using a technology which allows us to examine the activity of over 700 human regulatory genes at one time. Once we have identified the regulatory genes which appear to distinguish a high-risk and low-risk type of LCIS we will see how well these regulatory genes can separate a different, larger group of LCIS specimens into two distinct risk subgroups. We will then compare the two subgroups created by our regulatory gene sets with subgroups we have created from a larger gene analysis, where we studied the activity of over 15,000 genes, to see if the two technologies separate the same LCIS lesions into the same subgroups. By using these two different types of gene sets to study the same LCIS lesions we will be able to learn more about how certain genes work independently and together to lead to the development of invasive breast cancer. Finally, we will confirm our findings by determining if the genes we have identified are turned on or off in historical patients with LCIS that have or have not developed invasive breast cancer. This research is significant because it will lead to a method of risk stratification which can be used in clinical practice for patients with a diagnosis of LCIS. A better understanding of an individual patient's risk for breast cancer will then allow physicians to make tailored prevention recommendations. Patients found to have the high-risk subtype of LCIS would benefit from chemoprevention and more aggressive

Pending Execution of Grant Agreements

surveillance strategies to allow for early detection and treatment, thereby reducing the risk of developing invasive breast cancer. Whereas patients with lower risk LCIS would be afforded the option of surveillance with reassurance that they are not at high risk.

PI Name: Paolo Serafini

Mechanism: Career Catalyst Research

Institution: University of Miami, School of Medicine

Application Title: Targeting Myeloid Derived Suppressor Cells and Regulatory T Cell to Improve the Efficacy of Anti-tumor Vaccine in a Spontaneous Model of Mammary Carcinoma

Abstract:

Breast cancer is the most frequent malignant tumor of women in North America. Standard treatments have improved the quality of life for women with breast cancer; however, the fact that 40% still succumb to disease highlights the need to identify new therapeutic targets and approaches to improve the survival chances. The high mortality is mainly due to the capacity of tumor cell to disseminate into the body rendering difficult their identification and impossible their surgical removal. Other conventional treatment such chemotherapy and radiation, only delay tumor recurrence without significantly improving the survival of women with breast cancer. In the last years many efforts have been spent to generate anti-tumor vaccines able to teach the immune system how to recognize and destroy both primary and disseminated tumor cells. These vaccines work very well in the preclinical settings when they are administered before injecting tumor cells into mice. All animals in fact, are able to reject the tumor. However, when the same vaccines are administered when the tumor is present, they fail to generate a protective immunity able to inhibit tumor growth in mice and in human. These contradictory results are explained by the capacity of tumor cells to shield themselves from the immunological assault by recruiting suppressive cells from the host. We and other identified these suppressive cell populations (named myeloid suppressor cells (MDSC) and regulatory T cells (Treg)) whose depletion or inactivation restore the host anti-tumor immunity. Although inhibition of either MDSC or Treg has been shown to partially restore the function of the immune system, nobody explored if a greater advantage can be obtained by blocking both populations. Moreover, until few years ago, inhibition of these suppressive cells was possible only with highly toxic drugs that when used into the clinic caused the death of many patients. Recently we discovered that FDA approved drug, commonly used to treat erectile dysfunction (Sildenafil, Viagra™ and Tadalafil Cialis™) inhibit the MDSC functionality and restore a functional immune response able to recognize and attack the tumor. In the last, years another compound has been developed and is currently approved by the FDA for the treatment of coetaneous T cell lymphoma since it recognize and kill the neoplastic cells. This compound called Ontak, kill also the Treg, and clinical trial shown is efficacy in improving the antitumor immune response. However, some concerns have been raised by the possibility that such treatment can kill also the “good” Treg that protect our body from autoimmune disease. We are developing a new strategy that seems to destroy specifically the bad Treg that protect the tumor without affecting the “good” one. With this proposal we want to determine if by using Viagra, Ontak, our new strategy or a combination of these therapies can improve the efficacy of anti-tumor vaccine. This study is going to be performed in a clinically relevant, spontaneous murine model of breast cancer (called Balb-neuT). Contrary to most murine model tumors in these mice tumor is not injected but spontaneously develops because of an oncogene always activated in their mammary glands (the gene Her2Neu that promotes cancer development and it is active in 25% of human mammary carcinoma). Tumor growth, progression and pathologic

Pending Execution of Grant Agreements

characteristics in these mice are really similar to the human counterpart. Moreover, as observed in the clinic, anti-tumor vaccines failed in these mice even if administered before the tumor is clinically detectable. Specifically, with the proposed project we want: 1) compare for efficacy and safety 3 different strategies able to kill Treg; 2) evaluate in a model where MDSC are genetically inactivated, if Treg depletion further improve anti-tumor immunity; 3) evaluate the synergistic efficacy of strategies in which the suppressive shield generated by the tumor is pharmacologically removed and the immune system is trained by anti-tumor vaccine to recognize and destroy the malignant cells. If our hypotheses are correct, a realistic opportunity for a rapid translation of our preclinical data into clinic will be opened. It will be possible, in fact, to design clinical trial in which immune interventions, using FDA approved drugs and clinically tested vaccine, will be coupled with surgical resection of primary tumors. The anti-tumor immunity generated will be for sure less toxic and tolerable and possibly much more effective than the standard therapy currently used to inhibit tumor recurrence and metastasis in women with breast cancer.

PI Name: Isabelle Bedrosian

Mechanism: Career Catalyst Research

Institution: M.D. Anderson Cancer Center, University of Texas

Application Title: DNA Damage as a Biomarker of Risk of Breast Cancer

Abstract:

The last decade has produced substantial improvements in treatments for breast cancer and new technologies have shown that breast cancer is a diverse disease with several different types of breast tumors that can be identified on the basis of gene analysis. Yet, despite this remarkable progress in our understanding of breast cancer and its treatment, few gains have been made in the area of breast cancer prevention. Although there are a number of reasons for this, the most important is that our understanding of what happens early in the cancer process that actually leads breast cells to turn cancerous remains poorly understood. Greater understanding of molecular changes that occur early in the cancer process would improve our ability to identify which patients are at greatest risk for developing breast cancer and will also provide new opportunities to generate novel therapies for breast cancer prevention. This is especially important since increase use of screening mammography for early detection of breast cancer is also leading to increasing numbers of women being diagnosed with non-cancerous abnormalities, some of which are felt to predispose to future breast cancer. Nonetheless, most women with these abnormalities, such as ductal hyperplasia or lobular carcinoma in situ, will never develop breast cancer. Current diagnostic and clinical tools do not provide a means of distinguishing which patients with these diagnoses will go on to get breast cancer. A second challenge has been that there are no effective therapies, even if women at highest risk for breast cancer could be identified. Although tamoxifen can reduce risk of developing breast cancer, the side effects are not inconsequential and this has resulted in poor acceptance of this medication for breast cancer prevention. Therefore, current recommendations for women with such non-cancerous, but potentially high risk, findings on mammography are haphazard and range from observation to removal of both breasts. There is clearly a need to identify the early molecular changes that occur in breast cells that leads to breast cancer. This would allow us to test women undergoing breast biopsies for these molecular changes and thus better determine which patients are truly at high risk for breast cancer in the future. Improved understanding of the early changes in breast cells turning cancerous would also open the door to developing new drugs that might target these initial molecular abnormalities that predispose to cancer. Recently it has been proposed that cancer causing genes can directly cause damage to the DNA in cells and as a result predispose these cells to cancer. In support of this theory, we have found in women with breast cancer, normal appearing cells next to cancerous cells, do in fact show signs of damage to the DNA. This raises the possibility therefore that damage to DNA may be an important early molecular change in breast cells undergoing conversion to cancer and that this DNA damage may be identified before the cells start looking abnormal. In this proposal we will build on our initial observation to rigorously test whether evidence of DNA damage in non-cancerous appearing breast cells can in fact identify which of these cells have molecularly started the process of transforming to breast cancer. First, we will be evaluating breast biopsy samples from patients for signs of DNA damage. These patients can be subgrouped into women who,

Pending Execution of Grant Agreements

based on clinical history have average, moderate or high risk for future breast cancer. We will then see whether the number of women with DNA damage in their tissues is different in these different risk groups and compare to the levels of DNA damage in women with breast cancer. We would expect if DNA damage is linked to future risk of breast cancer, then the proportion of women whose breast tissues show signs of DNA damage is going to be greatest in the women whose clinical history would place them at highest risk for future breast cancer. In order to further strengthen the link between DNA damage in breast cells and the development of breast cancer, we will also evaluate the impact of being able to repair DNA damage on the ultimate risk of breast cancer. Thus if measuring DNA damage alone in breast cells does not predict future risk of breast cancer, it may be because the risk of cancer from DNA damage is changed based on the ability to repair such damage. We will therefore measure DNA repair capacity in our patient groups and see if we can link DNA damage and DNA repair together to best define risk of breast cancer.

We expect that the results of our study if positive will help to address the current pressing needs to advance the field of breast cancer prevention. First, these results will help us improve on our current ability to identify using molecular analysis, which women are predisposed to developing breast cancer. Second, if evidence of DNA damage in breast cells is linked to increased future risk of breast cancer, then this marker can be used in prevention studies; interventions that reduce the amount of DNA damage in breast cells would potentially reduce the risk of breast cancer. And lastly, if DNA damage is a prerequisite step to developing breast cancer, then developing drugs that directly targets the process of DNA damage and DNA repair in breast cells may be a new strategy for the prevention of breast cancer.

PI Name: Thomas Westbrook

Mechanism: Career Catalyst Research

Institution: Baylor College of Medicine

Application Title: A Functional Genomic Approach to Discovery of Breast Cancer Therapeutic Targets

Abstract:

One of the most important advances in breast cancer treatment (and cancer treatment in general) has been the development of molecular-targeted therapies. These drugs, which inhibit the function of the very genes that promote breast cancer, are successful because cancer cells often rely on these aberrant genes for their continued growth and survival. Unfortunately, the majority of these “breast cancer genes” cannot be directly inhibited, highlighting the need for new strategies to rapidly identify breast cancer drug targets. Importantly, the aberrant activation of breast cancer genes also provides new stresses to cells, a phenomenon called “oncogenic stress.” Consequently, cancer cells become dependent not only on the breast cancer genes themselves, but also on pathways that temper this stress. Such pathways represent ideal therapeutic targets, because cancer cells (but not normal cells and tissues) become uniquely dependent on these pathways. While a few examples of these cancer-cell specific vulnerabilities have been described, to date there has been no unbiased way of identifying the most sensitive of these vulnerabilities in cancer cells. Herein, we propose a novel method to identify genes and pathways that are only required when a breast cancer gene is activated. This method relies on a new technology (called shRNA library) that we have developed which enables us to systematically interfere with each gene’s function in a rapid manner. We will use this technology to define genes that are essential only in the presence of the breast cancer oncogene c-MYC. Notably, c-MYC is hyper-activated in a class of breast cancers (~30%) that are particularly aggressive and have no effective therapy. In pilot experiments, we have already demonstrated success of this new strategy, and we are expanding our method to interrogate the entire human genome. Using this approach, we will identify genes that can be inhibited to specifically kill such c-MYC+ breast cancer cells, and test these genes as drug targets in models of human breast cancers. Importantly, this approach will also provide a roadmap for discovering therapeutic targets in other breast cancer subtypes.

PI Name: Glendon Zinser

Mechanism: Career Catalyst Research

Institution: University of Cincinnati

Application Title: Breast Adipose Bioactivates Vitamin D and Contributes to Cancer Prevention

Abstract:

Background: These studies will focus on the relationship between vitamin D and breast cancer. Vitamin D is a steroid hormone that can be obtained from our diet or produced in the skin after sun exposure. Active Vitamin D binds its receptor, the vitamin D receptor (VDR), and participates in the maintenance of calcium levels in the body. Active Vitamin D, by binding to the VDR, also participates in regulating the growth of cancer cells by inducing tumor cells to stop growing or even undergo cell death. In clinical studies, low blood levels of Vitamin D correlate with increased breast cancer risk, disease progression, and metastasis. VDR is present in all cells of the mammary gland and is found in 80% of human breast cancers. However, treatment with active Vitamin D is not feasible due to the adverse effects that a surplus of active Vitamin D has on calcium levels. Instead, the inactive form of Vitamin D if provided through dietary means or treatment dose can be converted locally in the breast to the active form of Vitamin D if provided to normal breast epithelial cells. Contrary to this localized therapy to regulate normal breast development, breast cancer cells do not maintain the ability to produce active Vitamin D.

Hypothesis: We hypothesize that breast adipose will store inactive Vitamin D, convert the inactive form to active Vitamin D, and re-lease signals to stop breast cancer cell growth.

Study Design: To test our hypothesis, we will use mice that have a normal VDR (WT) and mice that lack a functional VDR (KO). Reciprocal transplantation experiments will be performed where we take ductal epithelium from WT mice and place it in the adipose fat pad of KO mice and vice versa. These experiments will distinguish the growth inhibitory contribution of WT adipose on breast development. We will also remove WT and KO adipocytes and epithelial cells and grow them in the laboratory in the presence of inactive and active Vitamin D. In addition to using mouse cells, we will also use normal human breast epithelial and adipose cells to assess the ability of inactive Vitamin D to be converted to active Vitamin D via the adipose cells. We will use a breast cancer cell line to confirm that the adipose cells will convert inactive Vitamin D to active Vitamin D and release signals to inhibit cancer cell growth.

Benefits: The proposed studies are novel because they offer the first investigation into the contribution of Vitamin D signaling via breast adipose tissue to negatively growth regulate breast epithelium. The proposed research is significant, because it is expected to provide a new method by which Vitamin D signaling serves to inhibit epithelial cell growth and breast cancer initiation. Therefore, our work would offer an explanation as to why elevated inactive Vitamin D serum levels decrease one's risk for developing breast cancer. Furthermore, our work would lobby for an increase in the recommended dietary levels of Vitamin D, thus inhibiting breast cancer initiation in more individuals in the years to come.

PI Name: Mark Cohen

Mechanism: Career Catalyst Research

Institution: University of Kansas Medical Center

Application Title: Nanocarrier-based Targeted Chemotherapy for Improved Drug Delivery in Locally Advanced Breast Cancer

Abstract:

Breast cancer is the second leading cause of cancer deaths in women today after lung cancer. Breast cancers initially invade regional lymph nodes on their way to spreading throughout the body. One significant problem with current therapy is the side effects these drugs create throughout the body including healthy tissues. To date no chemotherapy is directed to specifically treat at risk lymph nodes in breast cancer. There is, therefore, a critical need to develop novel drug delivery methods allowing chemotherapy drugs to be retained in the lymph nodes and treat them more effectively.

Our objective is to develop an innovative approach to treating locally advanced breast cancer which has spread to the lymph nodes. We will combine standard drugs with tiny nanocarriers, much smaller than a single cell, which allow the drugs to travel mainly through the lymphatics when they are given as an injection under the skin to arrive at tumors in the breast and in lymph nodes which are involved with tumor. Delivering and keeping the chemotherapy drugs mainly in the lymph nodes where the disease has spread should decrease the side effects and toxicity these drugs cause to healthy tissues elsewhere in the body. Also since these drugs are absorbed in a more controlled manner, they can be given less frequently than standard drugs which are typically given through a vein infusion or injection. We also plan to test head-to-head whether our nanoparticle chemotherapy drugs delivered under the skin are better at treating advanced breast tumors and lymph node disease than those same drugs given normally without the nanocarrier through a vein. Our approach is a new and powerful tactic in treating locally advanced breast cancer. By proving these novel drug formulas and delivery method are less toxic and better than standard drug therapies in treating mice with advanced breast cancer tumors, we can move these drugs forward into human trials in 2 to 3 years to treat patients with locally advanced tumors. This treatment method and innovative drug therapy can be used before surgery in patients with large tumors, where the goal is to shrink the tumor and prevent further spread of the disease. This treatment would also have an important role in patients who develop locally recurrent disease, especially in the lymph nodes, since these nanocarrier-drugs are better at getting to and penetrating lymph nodes than standard chemotherapy drugs. Finally this drug therapy could be helpful in patients who have smaller breast tumors removed with surgery who refuse radiation therapy but want an option that would help control the disease and prevent recurrence. The successful completion of this project will serve as a strong foundation for establishing intralymphatically targeted chemotherapy as an exciting new option for patients in the treatment of breast cancer. In summary, this research will result in a new therapy for lymphatically metastatic breast cancer that could beneficially impact the lives of patients with both early and advanced breast cancer. If the nanocarrier drugs treat the local tumors better than the standard drugs without any added side effects or if they treat the tumors equally as well but with fewer side effects, these drugs would significantly improve our ability to treat these difficult tumors and provide patients with better options for

Pending Execution of Grant Agreements

treatment of their disease. This is specifically important in patients who have aggressive tumors that do not respond to hormone therapies where these nanocarrier chemotherapy drugs would have the most use.

PI Name: Kazuaki Takabe

Mechanism: Career Catalyst Research

Institution: Virginia Commonwealth University

Application Title: Sphingosine Kinase 1 as a New Target for Systemic Therapy against Breast Cancer – Development and Characterization of Novel Sphingosine Kinase 1 Inhibitor

Abstract:

Breast cancer kills patients because they develop metastases that spread all over the body. Localized early cancer can be controlled either by surgery or radiation, and rarely results in poor outcome. Therefore, systemic therapy is the key to reducing the mortality of breast cancer. Currently, combination chemotherapy with anti-estrogen therapy, is the standard systemic therapy regimen, and is effective to 90% of initial primary breast cancers. However, even among early-stage cancers, approximately 30% develop recurrence. Recently anti-Her2 antibody (trastuzumab) has been shown to improve survival in advanced metastatic breast cancers that express Her2. Trastuzumab also halves the recurrence rate and improves survival in early breast cancer with minimal side effect. Therefore, targeted therapy is an approach expected to improve outcome, although trastuzumab is limited to Her2 positive cancers, which includes only 20-25% of all breast cancer patients, and resistance to treatment can often develop. Since angiogenesis is one of the hallmarks of cancer and is the process that leads to enlargement of the tumor and metastasis, bevacizumab, humanized anti-VEGF antibody, was developed and used for breast cancer treatment. In combination with paclitaxel, bevacizumab significantly improved progression free survival, but failed to demonstrate overall survival benefit, and also increased side effects. Hence, new targeted therapies that inhibit angiogenesis are eagerly sought. Several labs, including ours, have recently found that the bioactive lipid mediator, sphingosine-1-phosphate (S1P), regulates many functions important for progression of breast cancer, including cell growth, survival, migration, invasion and angiogenesis (Takabe et al. *Pharmacological Reviews* 2008;60:181-195). In fact, it has been reported that S1P is a more potent angiogenic factor than VEGF. Therefore, it is expected that inhibition of S1P production will result in inhibition of its functions, should suppress the enlargement of the tumor, and the occurrence of metastasis. Moreover, SphK1, the enzyme that produces S1P, has been reported to be elevated in human breast cancers and its expression correlates with poor prognosis (Shida, Takabe et al. *Current Drug Targets* 2008;9(8):662-673). Collectively, these studies and others suggest that targeting SphK1 is expected to be a good candidate as a new treatment modality of breast cancer, yet potent and specific inhibitors of this enzyme were not previously available to examine this possibility. The hypothesis of this study is that SphK1 plays a critical role in breast cancer progression by production and export of S1P that then regulates many cellular functions important for cancer progression. This hypothesis will be tested by development of SphK1 inhibitors that demonstrate potent anti-tumor effects against breast cancer both in vitro and in vivo without significant toxicity. Our lab recently developed two potent, water-soluble, specific inhibitors of SphK1, SK1-I and SK1-I2. We have published that SK1-I potently killed leukemia cells taken from patients, and significantly reduced tumor size after they were implanted to nude mice. In preliminary studies, I have shown that SK1-I effectively suppresses cell proliferation, colony formation, migration, and induces apoptosis in multiple types of cancer, including breast, in vitro. We

Pending Execution of Grant Agreements

also have preliminary results that SK1-I suppress angiogenesis in a rodent animal model as well. Hence, SphK1 inhibitors are expected to become a novel targeted therapy against breast cancer that act by a completely different mechanism from previous targeted therapies.

This proposal will help move these inhibitors into human clinical trials. It is well known that the majority of newly developed drugs do not reach the market due to insufficient efficacy and toxicity. Therefore, it is not only important to perform solid preclinical studies to demonstrate potency and efficacy of a new compound, but in fact, a compound proved to be useful by one is more likely become a drug to be used for patients. These studies will aid in development of new therapeutics targeted to SphK1 as well as new approaches to enhance conventional cancer therapies. We will test SphK1 inhibitors not only in cell culture systems, but also in animal models, which will become a foundation of clinical trials. In addition, we have recently established a mass spectrometry method to directly measure endogenous levels of S1P, which has not been possible previously. Utilizing this technology, we will investigate the relationship between plasma S1P levels and severity of breast cancer and/or responses to conventional therapies. We expect that this data will support the hypothesis that overexpression of SphK1 leading to increased S1P production is an important factor in breast cancer progression and further assist us to develop effective treatment strategies to improve survival of advanced breast cancer. Based upon our preliminary data on leukemia and glioblastoma, it is very likely for our SphK1 inhibitors to show significant anti-tumor effect on breast cancer. Furthermore, due to the strong angiogenic property that S1P possess, we can expect our SphK1 inhibitors to act as potent anti-angiogenic agent. Since angiogenesis is essential for enlargement of the tumor, and the occurrence of metastasis, strong anti-angiogenic agent is expected to make the tumor become chronic, which I believe is the most realistic way to prolong advanced breast cancer patients' lives. In order to help the patients in need, we must carry out preclinical trials in animal models, to prove the efficacy and safety.

PI Name: Arvind Pathak

Mechanism: Career Catalyst Research

Institution: Johns Hopkins University, School of Medicine

Application Title: Developing Non-Invasive, in vivo Biomarkers to Guide Antiangiogenic Therapy in Breast Cancer using MRI

Abstract:

“Angio” means vessel, and “genesis” means creation or origin, thus “angiogenesis” is the creation of new blood vessels. This is an important natural process occurring in the body, both in health and in disease. For example, in the healthy body angiogenesis occurs for healing wounds and for restoring blood flow to tissues after injury. In females, angiogenesis occurs during the monthly reproductive cycle (to rebuild the lining of the uterus) and during pregnancy (to build the circulation between the mother and fetus). The healthy body controls angiogenesis through a series of "on" and "off" switches. The main "on" switches are known as angiogenesis “stimulating” growth factors, while the main "off switches" are known as angiogenesis “inhibitors”. When angiogenic growth factors are produced in excess of angiogenesis inhibitors, the balance is tipped in favor of blood vessel creation. When inhibitors are present in excess of stimulators, the process of angiogenesis is halted. The normal, healthy body maintains a perfect balance of these “on” and “off” switches. However, in many serious diseases such as breast cancer, the body loses control over angiogenesis resulting in the excessive growth of new blood vessels. This led scientists to hypothesize that tumor growth was dependent on angiogenesis, and switching it “off” with special “anti-angiogenesis” drugs could profoundly affect the quality of life of patients. Recently, it was demonstrated that such “anti-angiogenesis” therapies temporarily stabilize or “make normal” the typically disorganized blood vessel growth that accompanies cancer progression. By “make normal” we mean that these drugs temporarily improve blood flow and hence delivery of oxygen and drugs to the tumor. Finally, recent clinical trials in breast cancer patients have demonstrated that when “anti-angiogenesis” drugs are followed by standard therapies such as chemotherapy, it resulted in a marked improvement in patient outcome compared to either treatment used alone. The findings from these new clinical trials in breast cancer patients suggest that the temporary “normalization” of blood vessels with “anti-angiogenesis” therapies facilitates better delivery of conventional drugs, resulting in more efficient treatments and improved patient outcomes. These exciting findings have made it imperative to develop new tools for physicians to: (i) plan the dosing of these new drugs, (ii) obtain early signs of whether these drugs are working or not, and (iii) monitor large numbers of patients repeatedly with minimal discomfort and maximal safety. Currently, the most common method for assessing the effects of these “anti-angiogenesis” therapies is the tissue biopsy. This is an invasive procedure during which tissue samples are removed from the patient and examined under a microscope by an expert. Although the biopsy is standard practice, it is extremely uncomfortable for the patient, it is an invasive procedure that does not lend itself to repeated assessments, and provides only a static “snapshot” of breast cancer angiogenesis. This application seeks to develop and validate tools that will enable physicians to perform the patient assessments described above in a non-invasive, repeatable and safe fashion, with profound implications for patient management and outcome. In addition, we are proposing to develop tools that employ imaging techniques

and chemical agents already in clinical use, thereby ensuring their rapid adoption into the clinical setting following rigorous validation.

Our overall goal consist of three hypotheses: First, that it is possible to develop non-invasive, in vivo (i.e. in the living organism) markers (also called “biomarkers”) of angiogenesis using magnetic resonance imaging or MRI. Second, that it is feasible to develop non-invasive, in vivo biomarkers of tumor oxygenation and tissue death or necrosis using MRI. Third, that these non-invasive biomarkers will enable the safe and repeatable monitoring of the efficiency of an “anti-angiogenesis” drug in breast cancer. Each hypothesis will be tested in an animal model of breast cancer using magnetic resonance imaging (MRI). For the first hypothesis, we will track growth of tumor vessels in the live animal using an MRI method that requires administration of a special chemical known as a “contrast agent” that makes blood vessels more conspicuous when imaging. The contrast agent we will be using is called Feridex and approved by the FDA for clinical use. For the second hypothesis, we will image the oxygenation of the tumor in a live animal with blood acting as a “natural” contrast agent. We will use a special MRI method to image changes in oxygen carried by the blood as well as any tissue necrosis. Finally, for our third hypothesis we will track the biomarkers described above over two weeks in a group of breast tumor bearing animals treated with the “anti-angiogenesis” drug Endostatin, and compare them to biomarkers obtained from untreated animals. Once MRI is complete, we will sacrifice the animals and independently validate the MRI findings by microscopic examination of fluorescently tagged tumor tissue. We will then assess the ability of the imaging biomarkers to non-invasively track changes in angiogenesis, oxygenation and necrosis by comparing them to biomarkers assessed from microscopy. If seen to fruition, this proposal could radically change the existing clinical paradigm for tracking and managing anti-angiogenesis therapies in patients. The resulting imaging paradigm in conjunction with novel therapies has the potential to significantly ameliorate mortality from breast cancer.

PI Name: Kristi Egland

Mechanism: Career Catalyst Research

Institution: Sanford Research, University of South Dakota

Application Title: Autoantibody Profiling As a Novel Method for Early and Personalized Diagnosis for Breast Cancer

Abstract:

Public Abstract In the United States, one in eight women will develop breast cancer during her lifetime. Early detection of breast cancer allows a physician to treat the initial stage of the disease while the tumor is still confined to the breast, increasing the patient's chance for long-term survival. Once breast cancer spreads, or metastasizes, from the breast to other tissues of the body, therapies are limited, and there is no cure for the disease. While early detection is essential for initiating treatment before metastasis, currently used diagnostic methods for breast cancer have low sensitivity and are far from optimal. Moreover, acquiring tissue through an invasive biopsy or surgery is necessary for the currently available diagnostic markers, bearing a heavy burden on patients. The goal of Dr. Kristi Egland's research program is to develop a minimally invasive blood test for breast cancer detection that is highly sensitive, allowing for early diagnosis.

It is well established that breast cancer patients often produce antibodies to cancer cells. Cancer cells can have proteins that are present at much higher levels than in normal cells, and the immune system can sense these abnormal tumor proteins. Because it is equipped with an amplification system, the immune system can produce large amounts of antibodies to the abnormal proteins, even when the change in tumor proteins is too small to be detected by routinely used diagnostic tests. Therefore, by detecting the presence of antibodies to a particular tumor protein, rather than the tumor protein itself, we can achieve high sensitivity allowing for earlier diagnosis of breast cancer patients. This information on host-cancer interaction will give us a new insight on the patient's response to the disease and will allow a physician to better monitor a patient's response to therapy. Finally, it will be possible to perform the proposed assay as a conventional blood test without invasive procedures, thus easing the burden on patients.

We have laid down two foundation stones for this proposal. (1) We have identified a series of tumor proteins suitable for detection by anti-tumor antibodies in the proposed assay; (2) we have established a method to produce these tumor proteins in a format that will be detectable by anti-tumor antibodies present in the blood of breast cancer patients. Our hypothesis is that antibodies in the blood of breast cancer patients will recognize the tumor proteins made from our collection of genes. These antibody profiles can be used to accurately detect the presence of breast cancer on an individual basis.

First, we recently isolated a collection of genes, called a library, encoding membrane and secreted proteins from breast cancer cells. Subsequently, the genes in the library that were identical to genes used in normal essential tissues were removed. In order to determine which genes are represented in our library, we obtained DNA sequences of 25,277 of the genes. Secreted and membrane proteins are more likely to induce an antibody response than intracellular proteins. Therefore, the final result of our generated library is a collection of genes that encodes membrane and secreted tumor proteins that are abundantly present in breast cancer cells and should preferentially induce an antibody response in patients.

Pending Execution of Grant Agreements

Second, the Eglund laboratory has previously developed a robust method to produce these proteins in a form that is recognizable by human antibodies. To choose the most promising cancer protein candidates for the diagnostic assay, 43 membrane and 12 secreted proteins among the 100 most abundant library genes will be made in a form that mimics the protein structure in the body. We propose to screen these generated proteins with blood samples, which should contain antibodies against tumor proteins, from 300 women diagnosed with breast cancer before surgery, chemotherapy, radiation or other treatments. As a control, this information will be compared to blood samples from 300 normal, healthy women. The format for this assay is designed to yield multi-factorial data with the ability to recognize many different types of breast cancer, allowing for personalized treatment. At the end of this 3-year proposal, an assay will have been developed to detect antibodies that recognize the predicted breast cancer-specific proteins in the blood of breast cancer patients. A patient-related outcome should be achieved in less than 10 years. Detection of patient antibodies that recognize a panel of tumor proteins can provide an early, specific and personalized diagnosis for breast cancer patients, which will significantly improve outcomes and long-term survival of patients. Early diagnosis is essential in the fight against breast cancer and increases the likelihood of a woman being cured. Early detection is the means for a cure.

PI Name: Ron Bose

Mechanism: Career Catalyst Research

Institution: Washington University in St. Louis, School of Medicine

Application Title: Proteomic Identification and Reversal of Mechanisms of Herceptin Resistance in Her2/neu Positive Breast Cancer.

Abstract:

Her2-positive breast cancer is a common breast cancer subtype, representing 20-30% of all human breast cancer cases. It is defined by amplification of the Her2/neu gene and resulting overexpression of the Her2/neu protein. Prior to the advent of the Herceptin, a monoclonal antibody that targets Her2/neu, Her2-positive breast cancer carried a worse prognosis than other breast cancer types. Currently, the prognosis of Her2-positive breast cancer is considerably improved because of the availability of Herceptin and lapatinib, a kinase inhibitor drug that blocks the action of Her2/neu. However, cases of resistance to these therapies commonly occur and deaths due to metastatic Her2-positive breast cancer are still seen. Developing molecular markers to predict who will respond best to Herceptin, to lapatinib, or to combinations of these drugs with other compounds currently in clinical trials will be of great value in breast cancer treatment.

The Her2/neu protein is a protein kinase and activates a large and complex network of proteins within the cancer cell by altering the process of protein phosphorylation. Phosphorylation, the process of adding a phosphate group onto proteins, is a well known way for cells to activate or regulated biochemical pathways. Uncontrolled activation of protein phosphorylation is known to cause a number of human cancers, including Her2-positive breast cancer, chronic myelogenous leukemia, and lung cancers cases which have mutations of the epidermal growth factor receptor (EGFR). Biochemical methods to study phosphorylation in the cell include standard antibody based experiments, which can measure one or a few proteins at a time, or proteomic methods which can measure hundreds to thousands of proteins simultaneously. Proteomics is the global study of proteins within a cell or organism and is performed using well-established chemistry techniques.

In this proposal, we will measure the protein phosphorylation events in both Herceptin-sensitive and Herceptin-resistant breast cancer models. By comparing these sets of quantitative profiles, we will identify proteins that are activated by Her2/neu but fail to respond to Herceptin treatment. We will then test the role of these proteins in Herceptin-resistant cell lines in order to determine whether these proteins are causing resistance versus only correlating with resistance. We will identify, consent, and collect clinical records and data from patients with Her2-positive breast cancer receiving neoadjuvant (pre-surgical resection) chemotherapy at our institution, so that once these molecular markers are identified, they can be tested, retrospectively, in patient samples and correlated to patient's response rates to neoadjuvant treatment. We anticipate that the identification and follow-up laboratory based experiments on these molecular markers will take 3 years and the direct testing of these markers in patient samples would require an additional 1-2 years. Starting now to identify the appropriate patients and obtain their permission to access their medical records and prior biopsies would significantly speed up the progress of this research.

Pending Execution of Grant Agreements

The value of these types of molecular markers has been demonstrated in many cancers. The response of colorectal cancer patients to the antibody drug, cetuximab, is strongly influenced by whether their tumor has a mutation in the K-Ras gene. Use of molecular markers to define the presence or absence of estrogen and progesterone receptors and the Her2/neu protein has had a dramatic impact on breast cancer. Being able to predict which patients will best respond to a therapy results in patients being given the drug that is most effective for them and avoid drugs which will have low efficacy. Further, tailoring therapy based on more detailed knowledge of cancer's molecular alterations results in more cost-effective cancer care for society. This proposal will directly identify new markers for Herceptin resistance or response and will prepare to test these markers on breast cancer biopsies from patients being treated at Washington University School of Medicine. These markers can subsequently be incorporated into large cooperative group trials for rigorous evaluation on large numbers of patient biopsies. Currently, despite the best agents for Her2-positive breast cancer, metastatic Her2-positive breast cancer remains incurable. Additional clinical tools are needed to save the lives of women with Her2-positive breast cancer and this proposal will identify and help develop these clinical tools.

PI Name: Theresa Swift-Scanlan

Mechanism: Career Catalyst in Disparities Research

Institution: University of North Carolina at Chapel Hill

Application Title: Breast Cancer in African American Women: DNA Methylation Studies in Basal-like, HER2+, and Luminal A and B Subtypes

Abstract:

PUBLIC ABSTRACT Breast Cancer in African American Women: DNA Methylation Studies in Basal-like, HER2+, and Luminal A and B Subtypes Theresa Swift-Scanlan, PhD, RN Career Catalyst in Disparities Research Applicant Background: Although breast cancer incidence is lower for African American women as compared to Caucasian women, African American suffer higher rates of complications and death from the disease. African American women are far more likely to be diagnosed at an advanced stage, to be premenopausal at diagnosis, and to have breast cancer subtypes that behave aggressively such as the “basal-like” tumors. Here at UNC, we have identified subtypes of breast cancer with different clinical courses, and now are looking at how to prevent each type of breast cancer. The work of Millikan and colleagues (2007), provided the first clue that modifiable factors might relate to subtypes, For example, they found that women who breastfed multiple children for longer duration decreased their risk of basal-like cancer, while for the “luminal A breast cancer subtype, breastfeeding did not decrease risk. Traditionally, increases in the number of children and earlier ages at childbirth were thought to decrease risk of breast cancer. Now we know that these factors increase risk for basal-like tumors, but decrease risk for the luminal A breast cancer subtype.

This study, together with other research showing childbirth and breastfeeding modify gene expression, suggest that different breast cancer subtypes may be influenced in part by estrogen and other exposures that differently affect gene function. With methylation studies, we can now look at how these risk factors might modify gene function in a subtype specific manner. The rationale for this approach is that changes in DNA methylation can occur early in tumor development, and therefore, in addition to the potential to better define breast cancer subtypes, can also hold promise for use as markers of early detection and risk assessment.

Objective/Hypothesis: The central objective is to explore if DNA methylation, in combination with information on breast cancer subtypes and environmental exposures, can shed light on ways to reduce mortality in a population-based study of African American women with breast cancer. **Hypothesis:** Specific genes will show different patterns of DNA methylation according to breast cancer subtype and menopausal status. Additionally, it is expected that associations will be found between changes in DNA methylation of selected genes by exposures to estrogen, or alcohol, and/or tobacco.

Specific Aims: Serious health care consequences and costs are associated with both over- and under-treatment of breast cancer resulting from our inability to predict how each individual woman’s breast tumor will progress or respond to treatment. Therefore, the goal of this proposed research is to expand the current understanding of breast cancer subtypes through a two part study of DNA methylation changes in breast tumors that may also serve as markers of early detection and risk. Since the majority of tumors used in past studies to identify distinct subtypes were derived from Caucasian women, we now propose to test if

Pending Execution of Grant Agreements

similar and/or different associations can be found with DNA methylation in African American women by: 1.) conducting a pilot study to quantify DNA methylation in genes from breast tumors representing four major breast tumor subtypes; Luminal A, Luminal B, normal breast, HER2+ ER-, and basal-like, and 2.) validating the results from the pilot by quantifying DNA methylation in a large population-based study of 80 premenopausal and 80 postmenopausal African American women with breast cancer. Because the basal-like subtype of breast cancer is more common in premenopausal African American women than postmenopausal African American women, differences in DNA methylation will also be explored between these two groups.

Study Design: The methylation pilot study will be a retrospective, laboratory study of DNA methylation in 40 breast tumors previously collected from a hospital-based study that first identified breast cancer subtypes by gene expression patterns. The next phase will quantify DNA methylation in an ongoing population-based study of African American women with breast cancer.

Relevance: It is expected that this study will provide preliminary evidence to support DNA methylation as one mechanism influencing specific gene expression patterns observed across breast cancer subtypes. Because DNA methylation is reversible through medical drugs, knowledge of gene methylation may also inform future development of therapeutic targets for breast cancer treatment. The goal is to ultimately decrease breast cancer mortality in African American women by advancing understanding of how different risk factors are related and such interactions define breast cancer subtypes in order to develop markers of early detection or treatment.

PI Name: Abenaa Brewster

Mechanism: Career Catalyst in Disparities Research

Institution: M.D. Anderson Cancer Center, University of Texas

Application Title: Ethnic differences in the mutational status of the PI3K pathway and breast cancer outcome

Abstract:

PUBLIC ABSTRACT Although there has been a decrease in the death rate from breast cancer among white women in every U.S. state, the death rate among African American women has continued either to rise or remain level. It is possible that poorer breast cancer survival among African American women may be caused by different breast tumor biology. The phosphatidylinositol-3-OH kinase (PI3K) pathway controls many of the biological processes that lead to the development and growth of breast tumors. The pathway is turned on or “activated” by insulin and growth factors or by errors or mutations that occur in important genes. Past studies consisting of white women have shown that activation of the PI3K pathway in breast tumors is associated with poorer breast cancer survival and lower response to breast cancer treatments such as tamoxifen and herceptin. Unfortunately, activation of the PI3K pathway in the breast tumors of African American women has not been studied although 80% of African American women are overweight or obese and this is associated with having higher serum levels of insulin and insulin-like growth factors. New drugs are being developed to target the PI3K pathway as a way of improving the treatment of patients with breast cancer, but it is not known whether African American breast cancer patients will benefit from these new drugs. We hypothesize that high serum levels of insulin and growth factors will be associated with a higher likelihood of activation of the PI3K pathway in breast tumors, and that African American patients will have a higher frequency of PI3K pathway activation than white patients. We will test this hypothesis by measuring levels of insulin and growth factors in the serum of 250 African American and 250 white breast cancer patients and examining their breast cancers for several tumor markers that indicate PI3K pathway activation. We hypothesize that the breast tumors of African American patients will also be more likely to have mutations in genes involved in the PI3K pathway than white patients, and that this higher rate of mutations will contribute to their worse survival. We will test this hypothesis by examining the breast tumors of 1212 African American, Hispanic and white patients for mutations in key genes in the PI3K pathway, and we will investigate the relationship between the rate of mutations and breast cancer recurrence. The proposed study is important because ethnic differences in activation of the PI3K pathway may contribute to differences in breast cancer outcomes. The study results will have immediate relevance to breast cancer patients by determining the eligibility of African American and Hispanic breast cancer patients for research trials of new drugs that target the PI3K pathway. If there is a correlation between serum levels of insulin and growth factors and activation of the PI3K pathway, these markers may be used to monitor patients’ response to therapy with PI3K pathway targeted drugs. The study is highly relevant to the Susan G. Komen’s mission to reduce the mortality of breast cancer in African American women. We propose to contribute to this mission by identifying a biological factor that may contribute to ethnic differences in breast cancer prognosis and can be targeted for the development and evaluation of new breast cancer treatments.

PI Name: Ana Gonzalez-Angulo

Mechanism: Career Catalyst in Disparities Research

Institution: M.D. Anderson Cancer Center, University of Texas

Application Title: Chemotherapy Resistance in Hispanic and African American Patients

Abstract:

More than 184,000 women will be diagnosed with breast cancer in 2008 in the United States alone and more than 1 million new cases will be detected worldwide. Breast cancer is a heterogeneous disease. Currently, clinico-pathologic criteria are used to guide therapy decisions. However, it does not define tumor biology as tumors of the same grade and stage often behave very differently. Neoadjuvant systemic therapy (NST), or given the chemotherapy before surgery, is the standard approach to treat women with large and inflammatory breast cancers, and is now being used in patients with earlier stage disease (smaller tumors). It is associated with a number of advantages such as shrinkage of tumors improving the chances of breast conservation, but also allows doctors to tell how sensitive a tumor is to a given treatment, providing the option to modify it for tumors that do not respond. Furthermore, if at the end of treatment there is no invasive cancer left (pathological complete response (pCR)), it has been shown by a number of investigators to be an indicator for improved long-term outcome. On the other hand, patients with residual breast cancer after NST are at increased risk for recurrence and may have treatment-resistant disease, but no standard therapy exists for them, based on the lack of molecular or clinical data to support its benefit.

Despite reductions in mortality, there are survival disparities among women of different racial groups. Most of the studies look at ethnicity and socioeconomic factors and their correlation with outcome. A few of them have looked at breast tumor subtypes of known poor prognosis, for example, there are data showing that compared to white women, young African American women have a higher incidence of triple negative tumors which could account in part for the biological factor relating to poor prognosis. Molecular studies for breast cancer are done predominantly looking at tissues from the Caucasian population. Minorities do not participate actively in clinical trials and today we are seeing interesting breast cancer outcome differences in these populations.

Recently, attention has turned to proteomics, which offers a powerful tool in the post-genomic era. Proteins are the direct executors of cell function. By studying the differences in protein expression profiles among different tumors, we expect to discover protein fingerprints that lead us to a better understanding of the mechanisms underlying the aggressive behavior of these residual cancers considered resistant to treatment. This approach is also likely, to identify new therapeutic targets in patients for whom conventional therapy may be inadequate. We intend to do this by using a novel functional proteomics technique known as reverse phase protein microarray or RPPA on tumor samples from breast cancer patients that had residual disease after NST. We will use 100 residual breast cancer samples and 25 pre-treatment samples (from which we have matched residual cancer) to identify active signals in the cancer cells that can guide us to identify novel targets for treatment that can be tested in animal models and later in clinical trials so we can change management and the prognosis of these aggressive tumors.

Pending Execution of Grant Agreements

An understanding of whether the differences in outcomes are due to health care access or different biological nature of the disease would have profound public health policy impact. A clear understanding of the way breast cancer behaves in Hispanic and African American patients is needed. Indeed, the lack of laboratory and clinical studies of the effects of the genetic diversity of tumors in ethnic minorities on the response to therapy results in these receiving therapies designed for Caucasian patients potentially adversely affecting the outcomes for minorities. Further, in the absence of an understanding of the ethnic diversity of breast cancer, potential markers and targets specific to African American and Hispanic patients will be missed much to the detriment of this underserved population. This Komen for the Cure Catalyst Award on Disparities will provide critical new information that could rapidly impact and improve the outcomes for minority patients. Further, it will help me achieve my career goal of becoming a leader in breast cancer research and in particular how to improve the outcomes for underserved minority patients.

PI Name: Catherine Drendall

Mechanism: Career Catalyst in Disparities Research

Institution: Duke University Medical Center

Application Title: Development of Proteomic Signatures of Risk to Estrogen-Independent Breast Cancer

Abstract:

The lack of representation of African American women in early detection and prevention trials mirrors our inadequate understanding of the risk factors contributing to breast cancer in African American women. Additionally, the age-adjusted 6-year breast cancer-specific survival rate for young African American women is disproportionately lower (69% vs. 84%) compared to Caucasian women. Thus, new prevention strategies are needed to reduce or eliminate this mortality rate in African American population.

In this research proposal, we will investigate the biology of breast cancer initiation in young African American women and test for predictors of risk in the sisters and daughters of premenopausal African American women who have been diagnosed with breast cancer. Successful completion of these research aims will identify biomarkers of short-term breast cancer risk in premenopausal African American women and cell signaling pathways that can be targeted to prevent ER-negative or ER-independent (ER(-)) breast cancer in these women. One type of ER(-) breast cancers that tend to be more aggressive and prevalent in young African American women is basal-like breast cancer. The underlying mechanisms for the highly proliferative capacity of basal-like breast cancer cells that affect younger African American women are not well understood. The current treatment for this type of breast cancer is combination of chemotherapy regimens. However, a subset of the affected women remains resistant and at a high risk of developing metastatic breast cancer. By understanding the biology of breast cancer initiation and the loss of regulation of signaling pathways in ER(-) breast cancer, we can begin to identify existing or new targeted agents that will likely be effective against basal-like breast cancers. We have preliminary protein microarray data on breast epithelial cells obtained from high-risk African American women that suggests up-regulation of signaling proteins along the Akt/mTOR pathway. This pathway promotes cell proliferation and survival. The signaling proteins along this pathway are usually up-regulated by a process called phosphorylation. There are also preclinical and clinical data from other groups that implicate the loss of regulation of the Akt/mTOR pathway in breast cancer. In our proposal, we will test whether the loss of regulation of the Akt/mTOR signaling plays a role in the initiation and progression of ER(-) breast cancer in premenopausal African American women. First, we will test the distribution of protein phosphorylation during breast cancer initiation in premenopausal African American women. Second, we will determine whether the loss of regulation of the Akt/mTOR pathway predicts resistance to Tamoxifen, an anti-hormonal agent used for prevention of breast cancer in high-risk women. The breast epithelial cells that we will test in these studies will come from premenopausal African American women that are currently enrolled in our high-risk clinic or that we will prospectively recruit through our Patient Navigator Outreach Program. In summary, the information gleaned from our studies will contribute to the prevention of ER(-) breast cancer and reduction of breast cancer mortality rate in African American women.

PI Name: Heather Ochs-Balcom

Mechanism: Career Catalyst in Disparities Research

Institution: State University of New York at Buffalo

Application Title: Search for novel breast cancer susceptibility genes in pedigrees of African ancestry

Abstract:

In the U.S., women of African ancestry have higher incidence of pre-menopausal breast cancer and higher mortality compared to Caucasian women. Breast cancer is a complex disease, where risk factors and the burden on receptor negative tumors vary according to ethnic and racial groups. Women with a family history who are not BRCA1 or BRCA2 mutation carriers are confused about why their disease is shared with family members. This concern has been expressed within the Witness Project (WP), a national cancer outreach program for African American women, for whom breast cancer is associated with a mortality rate that exceeds that of other races. The consistent association of more severe breast cancer phenotypes (receptor negative) in women of African ancestry along with the lack of studies designed specifically in African American women is the basis for the proposal. The goal is to investigate genetic factors related to breast cancer disparities. The rationale for the proposed work is that examining shared genomic regions within families where there are multiple cases of breast cancer may reveal novel genomic regions unique to women of African ancestry that are important in breast cancer risk. We propose a community-based participatory research, mixed methods and multi-site study to optimize recruitment of families for a genetic study. To develop a community-based partnership, we will collaborate with the Witness Project to recruit African American women diagnosed with breast cancer and their unaffected relatives. We will also use focus groups to investigate incentives and factors related to participation in a family-based genetic study in this minority group. The aims are: 1) work with the Witness Project members to develop a process to optimize recruitment and participation by African American women; 2) determine to what degree breast cancer risk factors and tumor types are shared among relatives; and 3) identify new regions of the genome that may increase breast cancer risk in African American families. To our knowledge, this is the first large study designed specifically to search for new breast cancer genes in families of African Ancestry who are not segregating BRCA mutations. We are well-prepared to undertake the proposed research in view of the multidisciplinary nature of the research team that includes expertise in medical anthropology, community-based participatory research, breast cancer epidemiology, genetic epidemiology and statistical genetics. We will undertake the aims of the study in collaboration with the Witness Project which is committed to developing a research agenda. This research will shed light on the genetics of disparity and will be more successful through the partnership with the Witness Project and its member community.

PI Name: Adrian Krainer

Mechanism: Post Doctoral Fellowship

Institution: Cold Spring Harbor Laboratory

Application Title: Role of Alternative Splicing in Mammary Epithelial Cell Transformation

Abstract:

Cancers are very complex diseases in which a large fraction of cellular processes are deregulated. Understanding cellular processes and molecular interactions is necessary to understand how cancers are initiated and to establish new ways of treating cancer. In Professor Krainer's laboratory, we work on "splicing", a critical step in gene expression, which determines how DNA instructions are cut and pasted together at the level of RNA intermediates to form final templates for the production of proteins. Like other cellular processes, splicing has been shown to be disrupted in cancer cells. These abnormalities can come from mutations in parts of genes that are important for splicing of their RNA copies, or from changes in the expression of proteins involved in splicing, called splicing factors. Recently, our laboratory has shown that several known splicing factors are present at higher-than-normal levels in some tumors, including colon, lung and breast cancers. Moreover, laboratory cultures of mouse or rat cells developed tumor-like characteristics when they were programmed to make higher-than-normal levels of a specific splicing factor known as SF2/ASF. However, the precise role of SF2/ASF, as well as that of other splicing factors, in cancer of various cell types is still to be determined. Several studies have suggested a role for splicing factors in the development of breast tumors in human and mice. Thus, I will follow up these exciting discoveries from our laboratory, and study the role of splicing factors in human breast cancer development. I will determine if splicing factors are involved in the initiation and maintenance of the cancer characteristics of mammary cells. I will first test if the expression of higher-than-normal levels of splicing factors can force normal human mammary epithelial cells to acquire the characteristics of cancer cells. I will use a new laboratory cell culture model that permits cells to be cultured in three-dimensions in a gel-like matrix. Instead of forming a flat cell monolayer, mammary cells form a spherical structure, which better mimics the cell environment seen in living organisms. Usually those cells form a very organized structure, a sphere with a hollow lumen, but when known cancer genes are expressed, this structure becomes highly disorganized. Thus, the cancer potential of new genes can be tested by analyzing the organization of those structures, as well as by monitoring their size, their growth rate and several other characteristics. We can also determine if any known cellular pathways, that is, sequences of events in which molecules affect one another in turn and lead to different cellular processes, are disrupted in these cells expressing higher-than-normal levels of a splicing factor. We will also look for new targets of those splicing factors, that is, genes which are affected and change their expression in response to those splicing factors. To do so, we will sequence all the genes expressed in cells expressing higher-than-normal levels of splicing factors and compare them to normal cells. We will also test if those splicing factors can induce cancer in cooperation with other known cancer genes. We will test if those cells can form tumors in laboratory mice, which would indicate that they can not only work in a cell culture dish, but also in a whole living organism.

Pending Execution of Grant Agreements

In parallel, I will analyze available breast cancer cell lines, originally established from breast cancer patients tumor biopsies, for the expression of splicing factors. I will look for cells expressing higher-than-normal levels of splicing factors, and I will block the expression of specific splicing factors to test their involvement in tumor maintenance. I will try to see if blocking highly expressed splicing factors or their target genes can force the cancer cells to lose some of the cancer characteristics and revert to normal mammary cells.

Alternative splicing is a key control point in gene expression, and it is now clear that it is also a process whose misregulation in cancer can contribute significantly to malignancy by regulating the expression of various cancer genes. This study will allow me to assess the role of splicing factors in breast cancer initiation and tumor maintenance. By identifying splicing factors involved in cancer and their specific targets, I hope in the future to be able to contribute to the development of alternative cancer therapies based on modulating the expression or activity of these factors or their targets.

PI Name: C. Kent Osborne

Mechanism: Post Doctoral Fellowship

Institution: Baylor College of Medicine

Application Title: Stress kinase signaling pathways in the development of hormonal resistance in breast cancer

Abstract:

Breast cancer is the most frequent form of cancer and the second leading cause of death for women in the US. It has been estimated that over 180,000 new cases will be diagnosed and over 40,000 women will die from breast cancer in 2008 in the United States. The majority of breast cancer patients will receive endocrine therapy as part of their treatment. Endocrine therapies, such as tamoxifen or the aromatase inhibitors, work by blocking the activity of a specific receptor within the cell, the estrogen receptor, which is able to drive tumor growth and progression. Unfortunately, for reasons that researchers do not fully understand, not all patients who are candidates to receive endocrine therapies will respond to these treatments, and even initially responding patients can develop resistance to endocrine therapies and die. These treatment failures account for thousands of breast cancer deaths each year among women all over the world. My mentor's laboratory has been working for almost 30 years on mechanisms leading to endocrine resistance in breast cancer. Preliminary data from our laboratory and from other groups show that activation of specific molecules belonging to the so called "stress-signaling pathways" within the tumor cells can affect the activity of the estrogen receptor and lead to the development of endocrine resistance. These molecular pathways are usually activated in normal cells in response to different stressors and are needed for the cell to respond properly to adverse environmental stimuli. Our hypothesis is that breast cancer cells could exploit these pathways to gain advantage on other cells and survive the endocrine therapy, thus resulting in tumor progression despite the treatment. To study the role of stress-signaling pathways in the development of endocrine resistance in breast cancer, we will first test whether the activation of these pathways can modify estrogen receptor activity in cultured breast cancer cells. Then we will perform additional experiments with the aim of understanding the molecular mechanism underlying the interaction between stress-signaling pathways and estrogen receptor. Finally we will use an animal model in which we allow human breast cancer cells to grow as tumors in mice and we will impair the function of stress-signaling pathways within the cancer cells to observe whether there is any change in the response to endocrine treatments. To do this we will use a genetic approach as well as new drugs that have been designed to specifically inhibit key components of stress-signaling pathways. These drugs are now in pre-clinical development but will soon be available to be tested in clinical trials. If our experiments confirm our hypotheses, these drugs could be immediately tested in the right patient population and could offer a new therapeutic tool to ultimately reduce the emergence of endocrine resistance and, thus, mortality from breast cancer.

PI Name: Paraic Kenny

Mechanism: Post Doctoral Fellowship

Institution: Albert Einstein College of Medicine at Yeshiva University

Application Title: TGF-alpha: A key molecule in basal breast cancer?

Abstract:

Breast tumors have been traditionally categorized based on the presence of certain key proteins including the hormone receptors for estrogen (ER) and progesterone (PR), and the overexpression/amplification of the HER2 oncogene. Recent advancements in breast cancer gene expression analysis allowed us to further refine these groups into 5 subclasses: two ER-positive subtypes (Luminal A and B), two ER-negative subtypes (Basal- and Normal-like) and the HER2 overexpressing subtype. Of these, the Basal-like subtype has the poorest prognosis, in part due to the absence of ER, PR and HER2 which are the targets of the most successful therapeutic approaches used in breast cancer therapy today (Herceptin, Lapatinib, Tamoxifen and aromatase inhibitors). These “triple-negative” tumors are also more aggressive, have increased local invasion and distant metastases and a higher mortality rate. Among breast cancers, basal breast tumors represent a distinct clinical entity; however, they are presently not treated any differently than other ER-negative subtypes. As these tumors do not respond well to targeted approaches, for these patients the only systemic therapeutic option is chemotherapy. A better understanding of the biology of this disease is needed to improve patient survival rates to be comparable to that of patients with other types of breast tumors.

Transforming Growth Factor-alpha (TGF-alpha) is a member of the Epithelial Growth Factor family (EGF) and it is a principal signaling protein in controlling cell proliferation. Our preliminary data indicate that TGF-alpha mRNA is highly expressed in basal breast cancers and that it confers significant motility to basal breast cancer cells in traditional two-dimensional cell culture. We will use three complementary approaches to explore the role of TGF-alpha in basal breast tumor invasion.

Firstly, we will seek to confirm our mRNA level findings to see whether the TGF-alpha mRNA produces a functional TGF-alpha protein in breast cancer by utilizing human patient tumor samples in a tissue microarray format which will allow us to test tumors from 245 patients in a single glass slide. We will also test these samples for the activation status of key members in the intracellular signaling pathways triggered by TGF-alpha, which will tell us if TGF-alpha is functional in these tumors. Furthermore, we will test whether high TGF-alpha levels are correlated with poor outcomes and overall survival in these patients. Secondly, we will use advanced three-dimensional culture models (that much more closely resemble the physiological environment of the tumor cells than traditional two-dimensional cell cultures) to examine cell invasion. This system will be used to directly investigate the contribution of TGF-alpha to basal breast cancer cell motility and invasion using cell lines that we have already confirmed to have high TGF-alpha expression. The expression and activation of TGF-alpha will be blocked in these cells and the motility and invasion compared to untreated cells. Thirdly, we will directly assess the effects of TGF-alpha on basal breast cancer cell motility using intravital microscopy, an advanced technique which allows us to see individual tumor cells in a human cancer cell line growing in a mouse as they invade and metastasize. Our basal breast cancer cell lines will be transplanted into mice to form

Pending Execution of Grant Agreements

tumors. The expression and activation of TGF-alpha will be blocked in these tumors and the motility and invasion compared to the tumors with high TGF-alpha content by directly imaging the tumor in the live animals.

Basal breast tumors have a significantly higher recurrence rate, leading to shorter disease-free and overall survival compared to all other breast cancer subtypes. Women with these tumors are very poorly served by existing therapeutic approaches and, until the biology of the disease is better understood, the prospect of effective targeted treatment strategies remains remote. We have previously determined that TGF-alpha mRNA-levels in basal breast tumors correlate with poor prognosis and the proposed work will provide detailed insight into the mechanism by which activation of TGF-alpha signaling contributes to the progression of the disease. As the mortality of this disease stems primarily from the high levels of local invasion and widespread metastasis, all a function of tumor motility, we focus specifically on the role of TGF-alpha in this process.

We anticipate that currently available specific receptor inhibitors and the inhibitors blocking the activation of TGF-alpha presently entering clinical trials will prove useful in the control of this disease and that the proposed work will provide a rationale for the use of these drugs to improve outcomes in women with basal and triple-negative breast cancers.

PI Name: Howard Chang

Mechanism: Post Doctoral Fellowship

Institution: Stanford University, School of Medicine

Application Title: Noncoding RNA, Polycomb and Breast Cancer Progression

Abstract:

Non-technical overview of the research topic and relevance to breast cancer:

Metastasis is the most fear aspect of cancer by which tumor cells spread from primary tumor to distant organs and grow relentlessly. Breast cancer can spread to other locations of the body at seventy percent of the time; tumor cells go through the blood stream or the lymph system to the lungs, bones, brain, liver, or skin. Since the metastatic cells are extremely versatile in their ability to adapt to different cellular and environmental conditions and are frequently resistant to conventional therapies. The main barrier to the treatment of metastases is that metastatic tumor cells are biologically different from primary tumors. Therefore, understanding the pathogenesis of metastasis on the systemic, cellular and molecular levels is our major focus. We found a novel noncoding RNA, HOTAIR, which is upregulated specifically in aggressive breast cancer cells. Unlike other messenger RNAs, noncoding RNAs do not encode proteins. It is estimated that over two third of human RNAs are noncoding RNAs but their functions are poorly understood. Recent observations also strongly suggest that noncoding RNAs contribute to complex networks needed to regulate cell functions. We first found that the expression of this novel noncoding RNA, HOTAIR, may inhibit the function of a metastatic suppressive gene. Our goals are to dissect the function of HOTAIR during breast cancer metastasis, and examine whether it can be used as a novel prognostic tool for metastasis and patient outcome.

The question(s) or central hypotheses of the research:

We hypothesize that high levels of a novel noncoding RNA, HOTAIR, mediate gene silencing complex function to initiate breast cancer metastasis by inhibiting a tumor suppressor, HOXD10, expression. Additionally, we will validate whether it can be developed as a novel prognostic tool for clinical usages.

The general methodology:

We will characterize the biological role of HOTAIR during breast cancer metastasis by expressing HOTAIR, and examine whether it causes cell invasive behaviors. We will also ascertain the molecular mechanism of HOTAIR function by examining whether HOTAIR acts with gene silencing complex to inhibit a tumor suppressor, HOXD10 during breast cancer metastasis. In addition, HOTAIR expression levels in stage I and II breast cancers will be examined and correlated with clinical follow-up data in a panel of 295 patients. These results will reveal whether HOX ncRNA expression can be used as a novel prognostic tool for metastasis and patient outcome.

Innovative elements of the project:

We identify a noncoding RNA that may initiate breast cancer metastasis. Noncoding RNA plays an important role in global gene silencing, but poorly understood. The proposed studies will be one the first to dissect their roles in breast cancer pathogenesis. Further, they

Pending Execution of Grant Agreements

may lead to radically new ways to diagnose and treatments, focusing not on protein but rather on RNA as functional diagnostic and therapeutic targets.

The importance of the research to patients with breast cancer:

One of the problems in breast cancer is underlying poor prognostic metastatic process. Having a clear understanding of the mechanisms of breast cancer pathology and metastasis is essential for developing new treatments. Noncoding RNAs are important in regulating global gene expression but poorly understood. Hence, discovering the molecular mechanism of the role of HOTAIR, a novel noncoding RNA, in breast cancer development and metastasis may provide an entirely new class of targets for pharmaceuticals in the fight against cancer. Even before novel treatment modalities are developed, an understanding of HOTAIR noncoding RNA may have a significant impact on the quantity and quality of patients' lives by offering a new prognostic biomarker. We anticipate that HOTAIR will be useful as a prognostic tool, and its expression patterns can be used to guide treatment decisions towards highly aggressive therapies to maximize life in patients with poor prognoses while avoiding unnecessary discomfort in patients with good prognoses.

PI Name: David Sabatini

Mechanism: Post Doctoral Fellowship

Institution: Whitehead Institute for Biomedical Research, MIT

Application Title: Identification of Small Molecule Transporters Essential to Breast Cancer

Abstract:

This study aims to identify genes of a particular understudied subclass which when inhibited will prevent breast tumor formation. The class of genes that we endeavor to inhibit contains those proteins which exist in the membrane of the cell and permit small molecules to enter or exit the cell. These small molecule transporters are responsible for controlling access to the cancer cell of key nutrients required for cell growth and survival. Because cancer cells differ from normal cells in their nutrient requirements, we expect that there exist a set of these small molecule transporters which are specifically required for breast tumor cell survival. We are proposing two complementary approaches to identify small molecule transporters that are required for breast tumor formation, one that relies on the information that has been gathered by numerous other studies and one that will analyze each transporter individually and in a way that is not biased to any information that we currently have. Under the informed approach, we have analyzed over 100 studies published by others or data unpublished by our group. This analysis has allowed us to generate a score for each gene in the group based upon several criteria including the associations that these genes have to cancer in general and to various types of advanced breast cancer specifically. Genes known to be important in cancer have scored highly in our analysis giving us confidence that the unstudied small molecule transporters that also scored highly in our analysis may be important in breast cancer as well. Under the unbiased approach, we intend to use the technology of RNA interference to specifically inhibit each small molecule transporter in the human genome in breast cancer cells collectively. These cells will then be grown in the mouse mammary fat pad and those which are able to grow will be isolated. By sequencing the DNA from these tumors, we will be able to determine which small molecule transporters, when inhibited, did not allow for the human breast cancer cells to grow. Once we have identified small molecule transporters by each of these methods, those genes which scored favorably will be inhibited in multiple different human breast cancer cell lines individually to verify the ability of each candidate to prevent breast tumor formation in multiple cell lines when grown in the mouse as described above. We expect that the small molecule transporters identified by this study can be used to design novel cancer drugs and treatments. Furthermore, this study would investigate a poorly studied set of genes with proven therapeutic potential, facilitating further study of this gene class.

PI Name: Weibo Cai

Mechanism: Post Doctoral Fellowship

Institution: University of Wisconsin at Madison

Application Title: Molecular Imaging and Therapy Targeting Breast Tumor Angiogenesis

Abstract:

In 2008, it was estimated that there are a total of 184,450 new breast cancer cases and 40,930 breast cancer deaths in the United States. Extensive studies have shown that vascular endothelial growth factor receptor (VEGFR) plays an important role during tumor development. Non-invasive measurement of VEGFR expression level can guide the treatment planning and monitoring for VEGFR-targeted therapy of breast cancer. Positron emission tomography (PET), a radionuclide-based imaging technique widely used in the clinic, has very higher sensitivity and it is quantitative. We have developed low molecular weight molecules (very stable under physiological condition) that can bind to VEGFR very tightly.

The overarching hypothesis is that radiolabeled molecules in this proposal will be stable enough in living subjects to allow for non-invasive, quantitative measurement of the VEGFR expression level in mouse models of breast cancer by PET scans, thus enabling effective monitoring of the therapeutic efficacy against breast cancer. Our long term objective is to develop versatile radiopharmaceuticals for breast cancer detection, therapy, and treatment monitoring. The ultimate goal of this proposal is to develop new PET tracers for future translation into clinical trials.

This proposal has three aims. In Aim 1, we will synthesize and optimize the PET probe and test them extensively in cells and in animal models of breast cancer. In Aim 2, we will evaluate the ability of the PET probe to quantitative measure breast tumor VEGFR expression level, this is the basis for Aim 3 in which we will use the PET probe to monitor the therapeutic efficacy against breast cancer and correlate the non-invasive PET data with histological analysis of the tumor tissue. We have extensive experience in PET probe synthesis, cell and animal studies, and non-invasive imaging of animal models, which makes these aims highly feasible.

Despite extensive investigation, there are no known predictive markers of VEGFR-targeted cancer therapy yet. Non-invasive measurement of molecular cancer markers such as VEGFRs may provide a means for much earlier diagnosis and predicting the therapeutic efficacy of new drugs. In the clinical setting, non-invasive measurement of tumor VEGFR level can provide an effective means to prospectively identify breast cancer patients who will benefit from VEGFR-targeted therapy and then stratify, personalize, and monitor the treatment to obtain optimal survival outcomes. The radiolabeled agents developed in this proposal will have broad applications in other cancer types, as well as many other diseases such as myocardial infarction and stroke. The knowledge gained in animal research can be readily translated to future clinical studies, heralding a new era of “personalized medicine” for cancer patient management.

PI Name: James Iglehart

Mechanism: Post Doctoral Fellowship

Institution: Dana-Farber Cancer Institute

Application Title: Understanding Breast Cancer Risk Conferred by the FGFR2 Single Nucleotide Polymorphisms

Abstract:

Breast cancer is a complex multifaceted disease that varies greatly among women in clinical behavior and treatment outcomes. New population-based studies show that different types of breast cancers have different risk factor profiles, suggesting that distinct susceptibility regions exist in the genome, thus possibly explaining this diversity of clinical outcomes. Recent advancements in genomic technology have allowed researchers to examine the whole genome for disease-specific alterations/variations on individual basis, thus gaining valuable insights into each individual's unique susceptibility to cancer. This research progress raises the possibility that combinations of newly discovered genetic markers can be used to improve the accuracy of cancer risk models, thereby improving cancer prevention programs. In fact, two genome-wide association studies have identified genes that predispose women to breast cancer. Single nucleotide variations in the gene called Fibroblast Growth Factor Receptor 2 (FGFR2) were found to be risk factors for breast cancer. The FGFR2 gene encodes a receptor tyrosine kinase involved in cell growth and differentiation. Interestingly, these single-base changes (known as single nucleotide polymorphisms or SNPs) occur in the intronic region of the gene, which usually does not have an effect on protein function.

This proposal is designed to understand how the FGFR2 gene risk region contributes to the disease. We have very exciting preliminary findings showing that nuclear protein complexes have differential preferences for risk vs. non-risk region in the FGFR2 gene. Based on these studies, we hypothesize that molecular phenotype of breast cancer risk conferred by FGFR2 SNPs may be explained by identifying critical proteins differentially bound to risk vs. non-risk alleles. Identifying these protein players and understanding the biology of breast cancer predisposition will result in development of more effective/more individualized approaches to breast cancer prevention and lead to identification of "druggable" targets associated with breast cancer risk profile.

PI Name: Susan Horwitz

Mechanism: Post Doctoral Fellowship

Institution: Albert Einstein College of Medicine at Yeshiva University

Application Title: The Role of MiRNAs in Mediating Resistance to Taxol®

Abstract:

Breast cancer is the most commonly occurring malignancy in women. Taxol® is FDA-approved and used extensively in the treatment of breast cancer. Taxol® binds to microtubules and stabilizes them, resulting in disruption of cell growth and the induction of cell death. However, many tumors do not respond to treatment with Taxol®, which is called intrinsic resistance, or they acquire resistance during treatment. The intrinsic and acquired drug resistance has been a major obstacle for advances in treating breast cancer. Studies have shown that drug resistance involves multiple mechanisms, including overexpression of the protein that can pump the drug out of cancer cells. Other mechanisms have been identified, and more remain to be discovered and characterized. Messenger RNAs (mRNAs) contain genetic information that can direct the synthesis of proteins. MicroRNAs (miRNAs) are short RNAs that regulate mRNA by suppressing protein synthesis. An increasing number of miRNAs are implicated in the development of cancer. The expression of miRNAs in drug resistant breast cancer cells has not been characterized, and could lead to a better understanding of the complexity of resistance and identify novel genes or pathways that have not been previously implicated in resistance. Therefore, we will identify which miRNAs are present in a unique panel of drug-resistant breast cancer cells compared with drug-sensitive cells. These cells have been selected to be resistant to Taxol®, and Ixabepilone®, both FDA-approved for the treatment of advanced breast carcinoma. The expression level of these miRNAs will be experimentally manipulated to test their effects on drug resistance, cell growth and cell death. By characterizing the repertoire of miRNAs implicated in breast cancer resistance, novel therapies can be devised and tested to target these miRNAs or the genes that they regulate.

PI Name: Naoto Ueno

Mechanism: Post Doctoral Fellowship

Institution: M.D. Anderson Cancer Center, University of Texas

Application Title: Development of EGFR Tyrosine Kinase Inhibitor as a Targeted Therapy in Inflammatory Breast Cancer

Abstract:

Inflammatory breast cancer (IBC), a rare but very aggressive form of breast cancer, is highly likely to metastasize, or spread to other sites. Because of its rarity (leading to little experience with treating it) and its resistance to treatment with standard chemotherapy drugs, IBC is associated with a much lower 5-year survival rate and higher risk of recurrence and metastasis than other types of breast cancer. It is thus critical for us to develop new strategies for treating IBC. Our goal is to create new, more effective treatments to improve the survival and reduce the metastasis of this disease. The epidermal growth factor receptor (EGFR), an enzyme expressed on the cell surface, seems to be crucial to the growth of tumor cells because it is expressed at high levels in various types of cancer, including breast cancer. Overexpression or the “turning on” of EGFR in cancer cells can enhance the proliferation and mobility of those cells and perhaps increase their ability to form metastases. Therefore, EGFR is being explored as a potential “molecular target” for cancer therapy. Erlotinib (marketed as Tarceva) is a small-molecule EGFR tyrosine kinase inhibitor (TKI) that is currently available for treating non–small cell lung cancer and pancreatic cancer. Erlotinib has not been particularly effective in noninflammatory breast cancer, but results of recent studies indicated that EGFR may be important player in the progression of IBC: EGFR overexpression was detected in 30% of IBC patients. High EGFR expression is associated with poor prognosis and increased risk of recurrence of IBC. These associations indicate that EGFR may be a therapeutic target in IBC.

We have already discovered that reducing EGFR expression inhibited IBC cell growth. Therefore, we hypothesize that blocking the EGFR by erlotinib inhibits tumor growth and metastasis of IBC. In this study, we plan to evaluate erlotinib’s effectiveness in preventing IBC tumor growth and metastasis. To achieve this, we have generated an animal model of IBC by injecting IBC tumor cells into the mammary glands of immune-deficient mice. IBC tumors formed in the animals very fast and also spread to the lungs. Therefore, this IBC tumor model efficiently evaluates the antitumor and antimetastatic activity of erlotinib. Several molecules are involved in the sensitivity of breast cancer to erlotinib. For example, Akt and ERK are important in EGFR-promoted cell proliferation. Identifying the way these molecules work will allow us to develop more effective EGFR-targeted therapies for IBC. Therefore, we will also study the role of Akt and ERK in erlotinib sensitivity by regulating their expression level. Knowledge of how they work will speed the development of new, clinically relevant therapies that will reduce the number of deaths among patients with IBC. We also discovered that expression of the protein called p27 changes when IBC cells are treated with erlotinib. p27 suppresses cell proliferation by inhibiting the progress of cells through their life cycle. We propose that p27 works through novel and potentially important mechanisms that underlie the resistance of IBC cells to anti-EGFR therapy. We will evaluate how this protein contributes to erlotinib sensitivity and will also evaluate the feasibility of

Pending Execution of Grant Agreements

using erlotinib plus molecules that target p27 to block tumor growth in cultured IBC cells and in our IBC mouse model.

The purpose of this project is to develop novel targeted therapies to improve the outcome of patients with IBC. This research is timely because erlotinib has been used successfully to treat lung cancer patients. It is unique because nothing has been reported to show that EGFR is a relevant target of IBC. Our work will provide results that will be useful in the clinic for understanding the role of EGFR in IBC progression and improving the effectiveness of EGFR TKIs for treating patients with IBC. Successful completion of this work will eventually extend the survival time of patients with IBC. The importance of this study is that it will be translatable to clinical treatment that will not only reduce the mortality of IBC patients but will also improve their quality of life by reducing the metastasis.

PI Name: Carlos Arteaga

Mechanism: Post Doctoral Fellowship

Institution: Vanderbilt University Medical Center

Application Title: Role of PIK3CA oncogenic mutations in HER2-mediated transformation and drug resistance in breast cancer

Abstract:

Background and hypothesis: Breast cancer is the second leading cause of cancer-related deaths in women in America. Approximately 178,000 women were predicted to develop breast cancer in last year. Yet, there have been many reasons to be optimistic. For example, in last decades, there has been an outburst of knowledge leading to better understanding of breast cancer pathogenesis (origination and progression of disease) and development of improved therapy. We now understand that based on specific clinical syndromes, molecular signature and biological behavior, breast cancers can be classified into different subtypes. Examples of these are hormone-dependent postmenopausal breast cancer and HER2-positive breast cancer. Based on the unique signature of a subtype, specific therapies have been tailored, the net result of which has been an overall reduction in breast cancer fatality. In HER2-positive breast cancer, the tumor tests positive for a protein called human epidermal growth factor receptor-2 (HER2), which promotes growth of cancer cells. Approximately 25% of all invasive breast cancers are HER2-positive. These are more aggressive than other types of breast cancers, less responsive to hormonal therapy and often related to poor patient outcome. Trastuzumab (Herceptin), a monoclonal antibody that specifically targets HER2, in combination with a chemotherapeutic agent, is FDA-approved for the first-line treatment of HER2-positive metastatic (cancer that spreads from the place at which it first arose as a primary tumor to distant locations in the body) breast cancers. Although clinically effective in killing HER2-positive cancer cells and decreasing the risk of recurrence by as much as 50%, many patients with HER2-positive breast cancers do not respond to or eventually quit responding to trastuzumab therapy, suggesting both inherent and acquired mechanisms of drug resistance. Recently (2007 March) a pharmacological inhibitor (blocker) of HER2, called lapatinib (Tykerb) has been approved to treat patients with HER2-positive advanced breast cancers. However, no patient data confirming development of lapatinib resistance is available yet.

Several studies have already reported or speculated on potential mechanisms of trastuzumab resistance. Many of these point to the aberrant activation of an intracellular signaling pathway, known as the phosphatidylinositol-3 kinase (PI3K) pathway. Activation of the PI3K pathway is central to cellular processes such as growth and survival, which if uncontrolled, give rise to cancer. In a large proportion of breast cancers (18-40%), overactivation of the PI3K pathway occurs through acquisition of mutations (change in the genetic code) in PIK3CA, the gene that encodes one of the subunits of PI3K. These cancer-associated mutations enhance the biological activity of PI3K. Interestingly, a significant number of HER2-positive tumors also harbor PIK3CA mutations and have invasive characteristics (e.g. lymph node positivity). Unpublished data from our laboratory also indicate that engineering HER2-positive breast cancer cells to express mutant PIK3CA confers resistance to lapatinib. Based on these observations, we suggest that acquisition of PIK3CA mutations is a potential mechanism to establish growth advantage as well as

Pending Execution of Grant Agreements

resistance to trastuzumab and/or lapatinib for HER2-positive breast cancers. We will use a variety of biochemical and molecular approaches to test this hypothesis. We will also develop mouse models for HER2-positive, PIK3CA-mutated, trastuzumab and/or lapatinib resistant breast cancer and determine whether small molecule inhibitors of PI3K alone or in combination with trastuzumab or lapatinib render the tumor sensitive to anti-HER2 therapy. Clinical impact: Resistance to anti-HER2 therapy poses a serious problem in treating HER2-positive breast cancers and a better understanding of the molecular mechanisms underlying drug resistance is needed to develop alternative therapeutic options for patients. The proposed research will help us determine whether activation of the PI3K pathway via acquisition of PIK3CA mutations confers resistance to anti-HER2 agents. This will in turn, help us 1) to screen HER2-positive patients for PIK3CA mutations in primary tumors and 2) to develop combination therapies for patients unresponsive to anti-HER2 agents, in which trastuzumab and/or lapatinib and PI3K inhibitors will be administered together. A substantial number of small molecule inhibitors targeting the PI3K pathway are currently under clinical development. Therefore, a combination therapy will likely improve overall survival of HER2-positive breast cancer patients.

PI Name: Sendurai Mani

Mechanism: Post Doctoral Fellowship

Institution: M.D. Anderson Cancer Center, University of Texas

Application Title: Prognostic Value of FOXC2 in Breast Tumors

Abstract:

The majority of deaths from breast carcinoma are due to the metastatic spread of the disease rather than the primary tumor. To metastasize, carcinoma cells, which are initially confined to the primary site by the continued expression of epithelial cell-cell adhesion molecules, reactivate a latent, embryonic program, called the epithelial-mesenchymal transition (EMT). Through EMT, epithelial cancer cells acquire mesenchymal-like traits, including motility and invasiveness, which seem to facilitate invasion and spread of breast cancer cells to distant sites within the body. Recently, we found that, in addition to increased motility and invasiveness, carcinoma cells that have undergone EMT also acquire properties of cancer stem cells, which can facilitate the colonization of distant tissues and enable the establishment of secondary tumors. Therefore, EMT would seem to endow tumor cells with many of the traits necessary for successful metastases in critical organs of the body. Indeed, metastasis is the ultimate cause of death for the majority of breast cancer patients. As such, identifying patients at risk of developing metastases would be of significant clinical importance. Since cancer cells that have undergone EMT exhibit stem cell properties and cancer stem cells are associated with tumor initiation, recurrence and metastasis, the identification of these cells within the primary breast tumors would be a strong indicator of poor outcome among breast cancer patients. However, there are currently no methods for specifically detecting cells that have undergone EMT in human tumors. A major reason for the inability to specifically identify cells that have undergone EMT in human patients is the significant similarity between cells that have undergone EMT and mesenchymal cells surrounding the tumor (e.g. fibroblasts). To identify markers specific to cells that have undergone EMT, we have previously performed gene expression analysis and found that FOXC2, a protein that has been implicated in tumor metastasis and is not expressed in most adult tissues, is upregulated subsequent to the induction of EMT by numerous EMT inducing signals. Furthermore, studies performed in vitro identified FOXC2 as a unique molecular marker for epithelial cells that have undergone EMT and found that FOXC2 expression can distinguish these cells from mammary fibroblasts in vitro. Therefore, we hypothesize that FOXC2 will serve as a good molecular marker for the identification of breast cancer cells that have undergone EMT as well as a prognostic indicator of aggressive human breast cancer. However, the relevance of FOXC2 to EMT, cancer stem cells and patient outcome still remains to be determined in vivo. To test this hypothesis, we will determine if FOXC2 is upregulated in transformed breast cells following the induction of EMT in vivo. In addition, we will test whether cells that have undergone EMT and express FOXC2 exhibit stem cell properties and increased metastasis in vivo. We will also determine whether the suppression of FOXC2 effects the traits associated with EMT and metastasis in vivo. Finally, our study will determine if FOXC2 expression in human breast tumors can predict poor clinical outcomes in patients with breast cancer. Most deaths among breast cancer patients are due to metastasis. Currently, prognostic indicators such as tumor size, lymph node involvement and hormone receptor status, are used to identify patients at high-

risk of developing metastasis. However, these current methods are not accurate enough to predict the clinical outcome in every patient and are poor predictors of metastasis. While survival rates are improving, there is a continuing need to accurately identify “good prognosis” subsets of women with early breast cancer who do not require adjuvant chemotherapy and / or hormonal treatments, and “poor prognosis” subsets who require either more intensive treatment or novel approaches to treatment. Therefore, it is important to develop better prognostic tools to identify these patients and treat them early. In this proposal, we will undertake a novel research study that aims to identify patients at high-risk of developing metastasis by utilizing the unique expression pattern of a key embryonic transcription factor FOXC2. The discovery of accurate indicators of disease will be critical for improving the clinical management of patients with breast cancer by identifying patients at high risk of aggressive disease, whom require intensive treatment regimes, and also, spare patients with low risk disease from the inconvenience, cost and side-effects of conventional breast cancer therapies. In addition to identifying the propensity of malignant cells to metastasize and aid the early prognosis of breast cancer, FOXC2 via its roles in EMT and CSCs, might also have potential as a candidate for the development of novel targeted therapeutics to prevent breast cancer development, metastasis and tumor relapse.

PI Name: Jason Weber

Mechanism: Post Doctoral Fellowship

Institution: Washington University in St. Louis, School of Medicine

Application Title: Role of p68 RNA helicase in the growth of breast epithelial cells

Abstract:

The majority of breast cancers express estrogen receptor (ER), a hormone-dependent transcription factor that regulates growth and proliferation by controlling gene expression in multiple tissues, including the breast. Endocrine therapies are a front-line approach for breast cancer treatment that relies on manipulation of circulating hormones to prevent ER from stimulating tumor growth and proliferation. While endocrine therapies are valuable treatment regimens, many women suffer relapse, characterized by estrogen-independent tumor growth and resistance to endocrine therapy. Thus, new strategies need to be developed to target molecules that support the growth and proliferation of breast cancer cells in the absence of estrogen. In other words, we want to identify the causes of relapse in order to support the development of effective therapeutics to overcome endocrine therapy resistance and tumor relapse in ER+ breast cancer patients. In collaboration with others, we have identified the gene for the p68 DEAD-box RNA helicase, DDX5 (17q24), as a member of an amplicon that is common in relapsed estrogen-receptor positive (ER+) breast cancer. The DEAD-box family of RNA helicases has been implicated in diverse cellular processes related to RNA metabolism. Our preliminary data has demonstrated a growth-stimulatory role for p68 in both mouse and human cells. The overexpression of p68 in ER+ breast cancer suggests that this RNA helicase may have a causative role in tumor progression.

We hypothesize that p68 stimulates growth in breast epithelial cells and facilitates estrogen-independent growth and survival of breast cancer cells. Two specific aims are proposed to test this hypothesis. Specific Aim #1 will test whether p68 has a growth-stimulatory role in cultured mouse mammary epithelial cells and human breast cancer cells. We will use viruses to either overexpress or knockdown p68 RNA helicase in order to determine its role on (A) estrogen-stimulated growth, and (B) estrogen-independent growth and survival of breast epithelial cells. In Specific Aim #2 we will develop a transgenic mouse model to drive p68 overexpression in the breast epithelium. Importantly, we have already generated and tested the targeting construct that will be used in this aim. Female mice with breast-specific overexpression of p68 will then be monitored for pathological signs of breast cancer. The aggressive nature of relapsed ER+ breast tumors makes the search for new therapeutic strategies of paramount importance. The experiments proposed in this application will elucidate the conditions in which p68 stimulates growth in breast epithelial cells, and in particular, ER+ breast cancer cells. Understanding the role of p68 RNA helicase in breast cancer, in the long term, will facilitate the development of novel therapeutic agents that can target its growth-stimulatory activity in tumors that are often resistant to standard therapeutic approaches. Pharmacological inhibition of p68 enzymatic activity and targeted down-regulation of p68 expression are amongst the potential avenues by which p68 function may be prevented. By functionally depleting ER+ tumors of an important RNA helicase stimulating estrogen-independent growth, proliferation, and survival, we may be able to eradicate an aggressive form of breast cancer.

PI Name: Leif Ellisen

Mechanism: Post Doctoral Fellowship

Institution: Massachusetts General Hospital

Application Title: p63-regulated micro-RNAs in human breast cancer

Abstract:

Public Abstract New approaches are needed in order to advance the goal of ending the suffering caused by breast cancer. Our laboratory has proven our ability to carry out basic research on breast cancer biology, and to translate our findings into new clinical trials that seek to improve the treatment options for women diagnosed with this disease.

Background: Our work involves a family of genes, called the p53 family, that have important functions in breast cancer progression. This family includes three “cousins”, termed p53, p63, p73. The p53 gene is involved in suppressing breast cancer and is therefore lost in many cases. In contrast, p63 promotes breast cell growth, and p73 is involved in the ability of chemotherapy to kill breast cancer cells. While p53 has been extensively studied in breast cancer, p63 and p73 have received less attention. Our recent work has shown that p63 and p73 are especially important in so-called “triple-negative” tumors, which are breast cancers that do not express estrogen receptor, progesterone receptor, or the Her-2 gene. Because these tumors lack these three factors, neither hormonal therapy nor Herceptin is useful for patients with this type of breast cancer. As a result, patients with triple-negative disease have a worse prognosis compared to patients with other types of breast cancer. Our work has shown that these tumors are particularly sensitive to a chemotherapy agent called cisplatin, which is less effective against other types of breast cancer. More importantly, we have demonstrated that testing for the presence of p63/p73 within the tumor cells might be a useful way to predict which patients will benefit most from cisplatin therapy. As a result of our work, a clinical trial is now underway that will test these predictions. Hypothesis and Methods: In order to understand the detailed function of p63 and p73 in breast cancer, I propose to identify and study a new class of genes that are controlled by p63 (which is itself a regulator of p73). These genes, called “microRNAs”, are known to be important for many properties of cancer cells. Based on the previous work in our laboratory, I hypothesize that p63/p73-regulated microRNAs will play critical roles both in breast cancer progression and in the response of breast cancer cells to therapy. The proposal involves three aims. In the Aim I, I will turn p63 “on” and “off” in breast cancer cells, then use a sophisticated microRNA “gene chip” to find out which microRNAs are controlled by p63/p73. In Aim II, I will test which of these microRNAs are actually present in human breast cancers, and which of them correlate with p63/p73 in the tumors, as predicted. I will then examine whether these microRNAs can predict which patients with triple-negative breast cancer will respond to cisplatin chemotherapy, by testing specimens from a clinical trial. This aim will therefore allow us to focus our studies on the most relevant and clinically important microRNAs. In Aim III, I will then carry out detailed molecular and biochemical studies to understand the precise function of these microRNAs in human breast cancer.

Objectives and Impact: The objective of these studies is to understand why p63/p73 are critical to breast cancer growth, survival, and chemotherapy response, by identifying p63/p73-regulated microRNAs and determining their functions. Identifying these microRNAs may have important implications for breast cancer patients. First, recent work suggests that

Pending Execution of Grant Agreements

microRNAs might be important biomarkers in human cancer. Therefore, in my study I aim to find microRNAs that might be useful predictors of treatment response for patients with triple-negative cancer. Finding such microRNAs would allow physicians to decide which patients might benefit from cisplatin therapy, and which patients would not benefit and should therefore try other alternatives. Second, because microRNAs are powerful regulators of many cellular functions, microRNAs themselves represent a new and attractive class of therapeutic targets. Identifying such microRNAs is therefore the first step toward realizing the promise of this new approach to breast cancer treatment. Relevance for patients: A major problem in breast cancer treatment is determining which therapy will actually work for a given patient. This study aims to find a new and more effective way to predict treatment response for patients with “triple-negative” cancer, one of the most resistant forms of breast cancer. Beyond predicting treatment response, this proposal seeks to uncover an entirely new way to treat breast cancer. I aim to find key microRNAs that can, in the future, be targeted to improve the cure rate and ultimately to eliminate this disease.

PI Name: Rekesh Jain

Mechanism: Post Doctoral Fellowship

Institution: Massachusetts General Hospital

Application Title: Optimizing the Penetration of Nanoparticles in Breast Tumors

Abstract:

Nanomedicine has provided new hope for detection and treatment of cancer. Nanoparticles can incorporate multiple diagnostic and therapeutic agents, but their large size restricts their transport/delivery through the micro-environment of breast cancers. Inadequate delivery of a nanoparticle like Doxil can limit the effectiveness of breast cancer therapy. We hypothesize that modifications of the interstitial matrix (gel-like space between cancer cells) can facilitate delivery and homogeneous distribution of therapeutics, and thus improve the effectiveness of the therapy. To this end, I will develop a mathematical model to predict the penetration of nanoparticles through the tumor interstitial matrix. The model will account directly for structural characteristics of the interstitial matrix and of the diffusing particle. Specifically, the model will incorporate the concentration of interstitial molecules and fibers, their local heterogeneity, and their charge as well as the size, shape and charge of the particle. I will perform necessary experiments to validate the model and subsequently, use the model predictions to determine what modifications in the interstitial space would allow the optimal delivery of nanoparticles. Finally, I will use the antiallergic/antifibrotic drug tranilast, which prevents the synthesis of collagen fibers, to modify the tumor interstitial matrix and I will evaluate its potential use for breast cancer therapy.

PI Name: Vassiliki Karantza-Wadsworth

Mechanism: Post Doctoral Fellowship

Institution: University of Medicine and Denistry of New Jersey, Robert Wood Johnson Medical School

Application Title: Functional Status of Autophagy in Tumors as a Determinant in the Treatment of Breast Cancer

Abstract:

Breast cancer is the most common female cancer, and the second most common cause of cancer death in women. Although several effective treatments have been developed, its cure is still a tremendous challenge, especially for metastatic disease. Novel and more effective therapeutic strategies against breast cancer are highly desired, strongly stimulating research to improve our understanding of the mechanisms responsible for breast cancer progression and responsiveness to chemotherapy and radiotherapy. Similarly to other cancers, breast cancer exhibits frequent genetic changes that allow tumor cells to bypass the mechanisms governing regulation of cell growth and cell death. Autophagy, or self-eating, is an evolutionarily conserved self-consumption process, which has been recognized as an adaptation process of normal cells to physiological stress, such as starvation and growth factor deprivation. Autophagy has also been shown to provide tumor cells with a survival mechanism to metabolic stress. Although constitutive activation of autophagy may ultimately result in cell death, restoration of regular growth conditions before a critical time point allows severely stressed cells to regenerate, indicating that autophagy may contribute to chemotherapy resistance and cancer treatment failure. In addition, about 50% of breast cancers exhibit lower than normal levels of the essential autophagy regulator beclin 1, indicating that defective autophagy may play an important role in breast cancer initiation, progression and treatment responsiveness. The studies in our laboratory have shed light into the mechanism by which intact autophagy suppresses breast cancer progression, whereas chronic autophagy defects promote tumor growth. We now want to define how the functional status of autophagy in breast tumors impacts treatment and how to best modulate autophagy to sensitize tumors to chemotherapy and radiotherapy. Autophagy-dependent tumor cell survival has recently emerged as a novel therapeutic target, as inhibition of autophagy is expected to deprive autophagy-competent cancer cells of a vital survival mechanism and, thus, promote tumor cell death. Therefore, we hypothesize that the functional status of autophagy impacts breast cancer treatment response and that autophagy modulation is a promising therapeutic target in breast cancer. Our first goal is to determine how autophagy affects the responsiveness of breast cancer to commonly used anticancer agents. Although autophagy activation is well documented in tumor cells in response to several anticancer agents, its role in treatment responsiveness is still unclear. Also, whether acute autophagy inhibition in autophagy-dependent tumors has the same therapeutic implications as chronic deregulation of autophagy in tumors with autophagy defects remains elusive. For this purpose, we will use our established autophagy-competent and autophagy-defective cell and mouse models to investigate the impact of acute autophagy inhibition and chronic autophagy deregulation on response to drugs commonly used for breast cancer treatment, such as doxorubicin, cyclophosphamide, paclitaxel, methotrexate, cisplatin, topotecan and tamoxifen. Autophagy will be inhibited by different

Pending Execution of Grant Agreements

methods in autophagy-competent models, and treatment efficacy will be evaluated by a variety of preclinical studies. Our second goal is to identify molecular signatures for the functional status of autophagy in breast cancer by comparing what genes autophagy-competent and autophagy-deficient mammary cells and tumors express. These investigations will provide a tool for determining the functional status of autophagy in mammary tumors and a way to stratify patients to appropriate treatments depending on individual tumor characteristics. Our long-term goal is to translate scientific findings into clinical work and provide solid laboratory data for the rational design of breast cancer treatments based the functional status of autophagy in tumors. Breast cancer patients living in NJ will particularly benefit from our work, as our preclinical studies will be used for the development of clinical trials at the Cancer Institute of New Jersey.

PI Name: Gabriela Chiosis

Mechanism: Post Doctoral Fellowship

Institution: Memorial Sloan-Kettering Cancer Center

Application Title: Pre-clinical development of tumor specific Hsp70 inhibitors in breast cancer

Abstract:

Breast cancer is a complex disease encompassing a number of distinct biological entities. A number of genetic alterations responsible for the malignant phenotype have been identified and a trend in cancer therapy has been to develop agents that “target” a single molecular alteration. These efforts have advanced the development of novel therapies such as Tamoxifen and Herceptin that have had success in patients with tumors dependent on the oncoproteins they target, the Estrogen receptor and the HER2 tyrosine kinase. At the metastatic stage however, tumors become highly heterogeneous and driven by additional and complex mechanisms. These multiple-activating oncogenic pathways stimulate the ability of cancer cells to proliferate, survive and metastasize, and in addition increase their resistance to chemotherapy. Faced with such heterogeneity and complexity, therapies that target a single activating molecule cannot overpower malignancy, and may be of little therapeutic benefit in metastatic disease, leaving these women with limited options. To overcome these limitations, we have developed small molecules that target the mechanisms triggering cancer development and resistance to therapies – the heat shock proteins. These proteins assist and abet malignant processes, such as proliferation, survival and metastasis, and allow for the development and existence of cancer. In addition, they help cancer cells build resistance to other therapies. They do these multiple tasks by regulating and keeping all the proteins that lead to malignancy in a functional state: these include, but are not limited to the estrogen receptor and the HER2 kinase. In addition, these proteins lock pathways that otherwise would lead to the killing of cancer cells by a process called apoptosis. Since the majority of therapies kill cancer cells by apoptosis, blocking of the process by heat shock proteins is cancelling their anti-cancer efficacy, and leads to treatment resistance. The first small molecules developed by our laboratory against heat shock proteins, target the heat shock protein 90, Hsp90. These molecules deplete breast cancer cells of their onco-proteins such as HER2 and ER, and have potent anti-cancer activity. The first molecule to stem from these efforts, CNF-2024, is currently in Phase 1 clinical evaluation and has demonstrated clinical responses in patients with HER2+ disease who have failed Trastuzumab. A second generation molecule, PU-H71, is more potent and has shown pre-clinically complete responses in both HER2 and triple-negative tumors. This molecule will enter clinical evaluation in patients with advanced breast cancers in 2009. While Hsp90 inhibitors are potent and promising additions to the anti-cancer armamentaria, we have come to learn that if we attack another heat shock protein, the heat shock protein 70, we can achieve the potency of Hsp90 inhibitors with the added benefit of inducing significantly higher killing by apoptosis of cancer cells. Besides abetting Hsp90 in keeping onco-proteins functional, Hsp70 has additional roles in locking apoptotic proteins and pathways, thus providing an explanation for the increased benefit of inhibiting Hsp70. Our efforts proposed here will focus on developing these Hsp70 inhibitors as novel breast

Pending Execution of Grant Agreements

cancer drugs, with the promise of delivering in the next decade a potent molecule for the treatment of metastatic disease.

PI Name: Dihua Yu

Mechanism: Post Doctoral Fellowship

Institution: M.D. Anderson Cancer Center, University of Texas

Application Title: Overcoming PTEN-loss Mediated Herceptin Resistance by Targeting Src Family Kinase

Abstract:

BACKGROUND AND RATIONALE: HER2 (ErbB2) is overexpressed in ~ 30% of human breast cancers. HER2 overexpression is associated with a highly aggressive tumor phenotype and poor prognosis. The HER2-targeted therapy, Herceptin (trastuzumab), significantly improves disease free survival. However, less than 35% of HER2 positive patients respond to Herceptin as a single agent. Herceptin resistance has become a devastating problem and there is an imposing need for new rational-based regimens to overcome Herceptin resistance. Multiple genetic and molecular alterations have been known to contribute to Herceptin resistance. We have previously demonstrated that phosphatase and tensin homolog (PTEN) activation is one of the most important mechanisms contributing to Herceptin anti-tumor activity. Loss of PTEN confers significant Herceptin resistance. Recently, when we reexamined the changes of cell signaling events in PTEN-loss cells, we found a hyper-activation of c-Src, another well known proto-oncogene protein. Non-receptor tyrosine kinase Src is a key signaling molecule at the convergent point of multiple growth factors related signaling. Src cooperates with EGFR family members (including HER2) synergistically promote tumor progression. In breast cancer, it has been demonstrated that Src activity is essential for HER2-mediated anchorage-independent growth, motility and survival. And Src inhibition is critical for Herceptin's anti-tumor function. Thus, this hyper-activation of Src under PTEN-loss condition suggests a cross-talk between PTEN and Src signaling. Facing the cells with PTEN deficiency, Herceptin not only loses a "executor" (PTEN) for its anti-tumor function, but also encounters a new "enemy" (elevated Src activity), who amplifies the HER2 signaling and antagonizes Herceptin's clinical efficacy. This abnormal high activity of Src in PTEN-loss cells (Herceptin resistant) makes Src an ideal drug target for potential combinatory regimen to overcome Herceptin resistance.

HYPOTHESIS: Based on preliminary observations, we hypothesize that PTEN may negatively regulates Src activity and combination of Src inhibitor and Herceptin could overcome PTEN-loss mediated Herceptin resistance and should bring significantly enhanced therapeutic benefits compared with either agent alone.

SPECIFIC AIMS AND STUDY DESIGN: We will use in vitro cell line models and in vivo orthotopic mouse model to test our hypothesis. We propose 3 specific aims: Aim 1) To evaluate Src activation in Herceptin resistant, HER2 overexpressing and PTEN-loss breast cancers cells. Aim 2) To investigate the molecular mechanisms of PTEN-loss mediated Src activation. Aim 3) To investigate whether targeting Src using specific Src inhibitor AZD0530 in combination with Herceptin treatment could overcome PTEN-loss mediated Herceptin resistance in vitro and in vivo. First, we will establish multiple paired PTEN wild type (wt) and PTEN-loss stable cells in endogenously HER2 overexpression cell lines and examine the Herceptin sensitivity and Src activation in paired those cell lines established. Second, we will explore the molecular mechanisms of potential direct/indirect negative regulatory role of PTEN on Src phosphorylation/activation. Most importantly, we will investigate whether

Pending Execution of Grant Agreements

targeting Src in combination with Herceptin treatment could overcome PTEN-loss mediated Herceptin resistance in vitro and in vivo. The potential sensitization effects of Src inhibitor in overcoming Herceptin resistance will be tested in in vitro cell line model. Then, we will use orthotopic mammary gland tumor xenograft model to investigate the potential synergistic anti-tumor activity of combinatory therapy of Herceptin and Src inhibitor.

IMPACTS OF THIS STUDY: This proposal is aiming to elucidate mechanisms responsible for Herceptin resistant, especially for PTEN-loss patient and design/test the novel combinatory approach to overcoming Herceptin resistance. Successfully completion of this project will significantly expand our current understanding of the mechanisms of Herceptin resistance and, most importantly, will directly impact on patient care. PTEN deficiency, one of the major genetic alterations responsible for Herceptin resistance, has been found in approximately 50% of breast cancer patients. There is imperative need to develop novel regimen to overcome PTEN-loss mediated Herceptin resistance. This proposed study will investigate molecular mechanisms of Herceptin resistance conferred by Src hyper-activation (Aim 1-2). This mechanism study will provide valuable insights of Herceptin resistance under PTEN-loss condition and lay a solid base to future guide the development of regimens for overcoming Herceptin resistance. More importantly, based on this mechanism of Herceptin resistance, the ultimate goal of this proposed study (Aim 3) is to seek effective combinatory therapies to overcome Herceptin resistance conferred by PTEN-loss. In this proposed study, we plan to use Src inhibitor AZD0530 in combination with Herceptin. AZD0530, as an effective anti-metastasis drug for advanced tumors, is currently under intensive Phase II clinical trials. In collaboration with our long-term collaborator Dr. Esteva in Department of Breast Medical Oncology, UT MD Anderson Cancer Center, the valuable findings derived from this proposed preclinical study could be directly and quickly translated into clinical trial settings. This translation will directly impact the clinical management of patients with HER2 overexpressing and PTEN-loss tumors and greatly benefit our patients.

PI Name: Hamid Band

Mechanism: Post Doctoral Fellowship

Institution: University of Nebraska Medical Center, Eppley Cancer Center

Application Title: Mechanism of Hsp90 Inhibitor-induced ErbB2 Degradation and anticancer effects in breast cancer

Abstract:

Nearly 40,000 women diagnosed with invasive breast cancer (over 200,000 in 2008) will lose their battle against cancer this year. While earlier diagnosis, more effective combination chemotherapy and the introduction of targeted therapies (such as Herceptin for Her2+ breast cancer) have improved survival, we are unable to impart “cure” in a majority of cases. One subset of breast cancer patients, accounting for a quarter to a third of all cases, is particularly unresponsive to conventional therapy and such patients carry a particularly poor prognosis of long-term survival. Tumors from these patients overexpress growth factor receptors, called ErbB2 (Her2) receptors. While recent advances in targeting these particular cancers with Herceptin and related agents have raised substantial hope, the inability of such therapies to produce clinical responses in all cases and toxicity associated with these treatments have emerged as important limiting factors. In order to overcome these roadblocks, it is imperative that we develop a better understanding of ErbB2 function and identify new molecular pathways that can be harnessed to produce more effective therapeutic response.

Studies carried out in the mentor’s laboratory, and other recent studies, have led to the hypothesis that candidate proteins called E3 ligases could aid in down-regulating the Her2/ErbB2 receptor. This proposal will use molecular and cell biological studies to demonstrate if E3 ligases indeed mediates down-regulation of the ErbB2 receptor independent of or in association with previously delineated mechanisms. Validation of the novel hypothesis proposed in this grant is anticipated to provide molecular basis for strategies that could enhance the efficacy of current treatments and implement the use of new drugs. Given the poorer prognosis of long-term survival among breast cancer patients with ErbB2 over-expression, our basic studies address a significant and urgent problem in breast cancer. Enhanced understanding of the basic biology of growth factor receptors and their role in breast cancer may also lead to insights of significance to breast cancer in general and possibly to other cancers.

PI Name: Vincent Cryns

Mechanism: Post Doctoral Fellowship

Institution: Northwestern University

Application Title: Targeting TRAIL Receptors in Metastatic Breast Cancer

Abstract:

Breast cancer is the most commonly diagnosed cancer in women in the United States and Europe and the second most common cause of cancer deaths. Metastasis is the final and frequently fatal stage of breast cancer when breast tumor cells spread to distant organs like the lungs, liver, brain or bones. Metastasis is a complex process involving many steps, including the movement and invasion of breast tumor cells out of the breast and into the blood and other organs. Unfortunately, radiation and chemotherapy are often ineffective against metastases. Therefore, new treatments for metastatic breast cancer are greatly needed. TNF-related apoptosis-inducing ligand (TRAIL) and human antibodies that bind and activate TRAIL receptors (TRAIL-R1 or TRAIL-R2) are promising cancer therapies because they trigger cell killing (apoptosis) in cancer cells but not normal cells. Both TRAIL and human TRAIL receptor antibodies are effective cancer therapies with little toxicity in animal models. A major advantage of the human TRAIL receptor antibodies over TRAIL is that these antibodies have well defined receptor targets and are more stable in the circulation than TRAIL. In fact, other human antibodies like Herceptin, which targets HER2, have had a major impact on breast cancer treatment. Lexatumumab (TRAIL-R2 antibody) and mapatumumab (TRAIL-R1 antibody) have been shown to be safe in patients with different types of tumors including breast cancer. Because one of the normal functions of TRAIL is to prevent metastasis, we hypothesize that activating apoptosis with TRAIL receptor antibodies will be an effective new approach to treat breast cancer metastasis. Our preliminary results suggest that we can trigger apoptosis in many highly metastatic breast cancer cell lines by treating them with lexatumumab (TRAIL-R2 antibody) or the combination of lexatumumab and chemotherapy. We have also shown in preliminary experiments that lexatumumab reduces lung metastases in an animal model of breast cancer. In this project, we will build on these results to determine the most effective combination of lexatumumab and chemotherapy that kills metastatic breast cancer cells and prevents metastasis in animal models. In aim 1, we will test multiple combinations of chemotherapy drugs and lexatumumab against metastatic breast cancer cells to identify the most effective combination. Our goal is to find a non-toxic dose of one or more chemotherapy drugs that triggers breast cancer cell death when combined with lexatumumab. We will also determine how these chemotherapy drugs increase cell death by lexatumumab. In aim 2, we will test promising combinations of lexatumumab and chemotherapy (determined in aim 1) against breast cancer stem cells from patients. These studies are important because breast cancer stem cells are resistant to chemotherapy/radiation and play a key role in metastasis, so effective metastatic treatments will have to eliminate these cells. We have set up conditions in our lab to grow breast cancer stem cells from patients as "mammospheres", clusters of tumor cells which contain breast cancer stem cells. We will test the effects of our combination therapy against these mammospheres to determine whether they kill breast cancer stem cells. In aim 3, we will test promising combinations of lexatumumab and chemotherapy in animal models of breast cancer metastasis. We have chosen two animal models of triple (ER/PR/HER2)

Pending Execution of Grant Agreements

negative breast cancer, a very aggressive type of breast cancer that affects young women and lacks effective treatments. We have added a fluorescent tag to these breast cancer cells so we track the cancer cells in living mice as they metastasize from breast tissue to the lungs and determine the effects of our treatment on metastasis. We predict that the combination of lexatumumab and chemotherapy will be most effective against metastases. Metastatic breast cancer is almost always a fatal disease because chemotherapy and radiation are usually ineffective at this late stage of the disease. Based on our preliminary studies, we believe that the combination of lexatumumab and chemotherapy will be an effective new approach to treat metastatic breast cancer. Our goal is that these experiments will lead to a clinical trial in patients with metastatic breast cancer that we will start planning with our oncology collaborators in the Robert H. Lurie Cancer Center during the last year of the grant. This goal is realistic because lexatumumab has been shown to be safe in patients and we are combining it with chemotherapy drugs already used to treat metastatic breast cancer or other cancers. Therefore, we expect to positively impact patients with metastatic breast cancer within the next ten years. These experiments will also provide me with hands on training in many breast cancer relevant fields, including apoptosis, cell signaling, pharmacology, cancer stem cells, and animal models of breast cancer. I believe this unique training program will prepare me well for a future career in breast cancer research leading my own lab.

PI Name: Paula Bates

Mechanism: Post Doctoral Fellowship

Institution: University of Louisville, School of Medicine

Application Title: Conjugated Tumor-Targeting Aptamers for Breast Cancer Imaging

Abstract:

>> **Background and Rationale:** The 5-year survival rate for breast cancer ranges from 98% when the disease is detected in its early stages to only 26% if the cancer has already spread to distant sites at the time of diagnosis. Hence, the number of deaths due to breast cancer could be reduced by better methods for early detection and by improved therapies that can eradicate relapsed, metastatic and recurrent disease. Any new imaging technologies will need to be minimally invasive, highly sensitive and extremely reliable; new therapies should have substantial selectivity for cancer cells compared to normal cells in order to minimize side effects. Both of these endeavors would be helped by the development of more effective “tumor-targeting agents”. These are molecules that when injected into a patient’s bloodstream will carry their cargo specifically to the breast cancer but will not be taken up in normal tissues. Thus, they can be useful for both imaging of breast cancer (where the cargo would be a material that can be seen on scans routinely used in cancer imaging) or therapy (the cargo would be a molecule that is toxic to cancer cells).

Hypothesis and Objectives: This proposal seeks to evaluate a new experimental anticancer agent named AS1411 as a potential tumor-targeting molecule. This agent is already being tested as a therapeutic agent and Phase I clinical trials involving 30 patients have indicated it has no serious side effects, but it has not yet been tested as an imaging agent. AS1411 is an “aptamer”—a short piece of synthetic DNA that binds specifically to a protein. The target protein for AS1411 is called nucleolin and it is present on the surface breast cancer cells and the blood vessels that feed the tumor, but not on the surface of most normal tissues. In mice models of cancer, molecules that target nucleolin have been shown to selectively accumulate in tumor tissues. Therefore we predict that AS1411 can be used to target breast cancer and we would like to test our idea in this project.

Study Design: Specifically, we plan to make and test two different types of molecules that consist of AS1411 linked to either radioactive groups or gold nanoparticles. The radioactive molecules will test whether AS1411 can be useful for positron emission tomography (PET), which is an imaging method used to detect primary and metastatic cancer. At present, PET imaging uses a radioactive type of sugar, which accumulates in the cancer, but is also taken up in some normal tissues. Potentially, radioactive AS1411 would be more specific for the tumor. “Nanomaterials” such as gold nanoparticles are revolutionary new types of technology that are very small (less than a millionth of an inch) with unusual physical, mechanical, electronic and optical properties. Relevant to this proposal, gold nanoparticles could be useful as new imaging agents to detect and treat breast cancer, especially if they can be directed specifically to the tumor. We will use several different imaging methods to test if our new molecules can be used to detect human breast tumors that have been implanted in mice.

Relevance to Breast Cancer: Standard mammography is a powerful screening tool, but has significant limitations: False positive results can occur causing needless worry and, even more seriously, false negatives are not uncommon in younger women due to their denser

Pending Execution of Grant Agreements

breast tissue. Complementary or alternative imaging techniques are therefore required. Optical mammography using near infrared (NIR) light to detect tumors is one such alternative that avoids additional exposure to X-ray radiation. Imaging techniques such as CT (computed tomography) and PET (positron emission tomography) are also useful, but all of these modalities would be greatly improved by the availability of contrast agents or radiopharmaceuticals that have highly specific uptake in breast tumors. In addition to molecular imaging, breast cancer targeting agents could also be used for therapy by attaching them to a variety of different ligands that destroy the cancer, either directly or inducibly (e.g. hyperthermia induction). These strategies should effectively eliminate the tumor without harming normal tissue.

Impact on Breast Cancer Patients: If the AS1411-linked imaging agents appear to be promising, they would be tested further in pre-clinical studies and clinical trials before being routinely used in patients. If successful, this could lead to new methods for imaging breast cancer within a few years. In addition, evidence that AS1411 is effective in targeting breast cancer would support the testing of AS1411, which is already in Phase II clinical trials in other types of cancer, as a new treatment for breast cancer.

Pending Execution of Grant Agreements

PI Name: Charles Perou

Mechanism: Post Doctoral Fellowship

Institution: University of North Carolina at Chapel Hill

Application Title: Molecular Characterization of Breast Tumors Subtypes in an Effort to Tailor Specific Cancer Therapies

Abstract:

Breast cancer is the most common cancer in women, affecting 1 out of 10 females. It is for this reason that this disease deserves much attention. However, it is a disease that is very difficult to treat due to breast tumors being very diverse in their natural history and in their responsiveness to treatments. The identification of distinctive molecular breast cancer subtypes may become a useful tool in treatment selection and development of new therapies. Our laboratories genomic characterization of human breast tumors has resulted in the identification of at least five distinct subtypes (Luminal A and B, HER2+/ER-, Normal Breast-like, Claudin-low, and Basal-like breast tumors (BBT)). These five subtypes each show their own unique biology, which highly correlates with distinct patient outcomes. Even with the advent of various mouse models of breast cancer, there have been difficulties in devising a model that accurately recapitulates human BBT. Despite significant advances, our molecular understanding of the causes of these subtypes, in particular BBT is still unclear. We hypothesize that for breast tumors to become BBT or any subtype of breast tumor that the first few genetics events that occur plays a major determining factor. BRCA1, TP53, and RB1 are all crucial tumor suppressor genes, and have been implicated in human breast cancer. In the case of human BBT previous work conducted in our lab has identified associations between BRCA1 mutations, TP53 mutations, and potentially the loss of function of RB1. The focus of my work over the next two years will involve the characterization of the biological diversity of human breast tumors, in particular BBT, using genomics, molecular genetics, and cell biology, in order to develop improved therapies that are specific for each tumor subtype. One of my primary goals will be to create improved “humanized animal models” of the aggressive basal-like subtype. We hope to better mimic in mice the genetic alterations seen with BBT formation by using CRE-LOX or gene knock-out technology, as oppose to viral oncogenes (cancer causing genes) which have many side effects. We then further hypothesize that these improved models will be better suited to compare to human BBT data, giving us additional insight into identifying specific mouse models of basal-like tumors that could then be used for chemotherapeutic studies and then to use these models to compare their sensitivity to various drug regimens that are clinically relevant (i.e. doxorubicin, paclitaxel, carboplatin, cytoxan, and combinations of these first, and then test combinations of chemotherapeutics with biologically targeted agents). Mouse models of breast cancer over the years have provided tremendous insights into the molecular aspects of how these tumors develop. Despite the many improvements in the reliability of various genetically engineered mouse (GEM) models, there are still criticisms to be made due to their lack of authenticity when compared to human disease. This is an important factor when it comes to the development and testing of targeted therapies. The proposed research utilizes GEM models with the hope that they could ultimately lead to advances in developing tailored treatment strategies for patients with various stages and

Pending Execution of Grant Agreements

subtypes of breast cancer. These advances could then lead to a breast cancer subtype specific reduction in mortality.

PI Name: Erkki Ruoslahti

Mechanism: Post Doctoral Fellowship

Institution: Burnham Institute for Medical Research

Application Title: Targeting Therapy-Resistant Tumor Vessels and Preventing Tumor Recurrence

Abstract:

Like all living cells, cancer cells need nutrients and oxygen to grow. Cutting off that supply is a way to prevent tumor growth. Therefore, several drugs have been developed to target the blood vessels that provide these essential elements to tumor cells. Among them, some are in clinical studies, and one has already been approved for the treatment of breast cancer. These therapies show promise, but it has turned out that they do not eliminate all tumor blood vessels. Some of these vessels remain intact and even see their function improved, leading to a long-term failure of the therapy. In this project, we propose to address the critical issues of tumor resistance to treatment and of tumor recurrence after therapies targeting tumor blood vessels. We plan to identify features specific for resistant vessels, and to make use of these features to destroy the remaining vessels. We will use mouse models of breast cancer where the cells are either of human and mouse origin and treat them with compounds that destroy tumor blood vessels. Then, we will use a method developed in Dr. Ruoslahti's laboratory, which consists of testing a large range of diverse molecules to find the ones that recognize the blood vessels that remain after the treatment. Having identified markers for the resistant vessels, we will use these markers to deliver blood vessel-destroying agents to the residual tumor vessels in treated tumors. This approach will lead to a better understanding of the fundamental mechanisms governing tumor growth and vessel resistance, and may produce compounds that can be used to improve breast cancer therapy. The specific targeting of therapeutic agents made possible by the technology we hope to develop will increase drug efficacy while reducing side effects. Combined with existing therapies, our new compounds could produce a total block of tumor growth.

PI Name: Jessica Tyler

Mechanism: Post Doctoral Fellowship

Institution: University of Colorado Health Sciences Center

Application Title: A novel epigenetic mark in breast cancer

Abstract:

Our chromosomes are made up of a basic repeating unit, called the nucleosome, which consists of our genetic material coiled up by eight histone proteins. The genetic material packaged into chromosomes is inaccessible and special machinery and modifications are required to loosen and move our nucleosomes out of the way to new positions on the DNA, so as to allow DNA-dependent biological processes to occur. Understanding how our nucleosomes are moved and loosened is central to gene regulation, genetic inheritance and repair of DNA damage, and understanding how these processes go wrong to cause cancer. We are studying a newly-discovered means to alter the nucleosome structure that involves chemical changes to the central portion of the histone proteins which results in breaking its interactions with the genetic material. Importantly, I have discovered for the first time that one of these specific chemical modifications of the histones occurs in humans and is greatly elevated in breast tumors.

Hypothesis: We predict that this specific chemical change to the fundamental chromosomal structure is elevated in breast cancer due to alteration of the activity or abundance of the enzymes that make or remove this chemical modification. Furthermore, we propose that the elevated levels of this specific chemical modification on the chromosomes plays a fundamental role in breast cancer tumorigenesis. This hypothesis will be tested by experimentally identifying the enzyme that removes this chemical change in humans via a candidate targeting approach – I have already identified the enzyme that adds this chemical change in humans. I will then examine whether the amounts of the enzymes that add or remove this chemical change are elevated or reduced, respectively, in breast tumors. I will then determine whether experimentally elevating the levels of this chemical modification in non-tumorigenic mammary cell lines causes them to become tumorigenic.

To our knowledge, we are the first scientists to discover this chemical change in humans, not to mention that it is greatly elevated in breast cancer. Therefore we are uniquely positioned to ask for the first time why this chemical change is more abundant in breast cancer patients and whether it is causative for breast cancer. As such, this project may reveal that this chemical change is a novel causative agent of breast cancer, and via my discovery of the enzymes that regulate the levels of this chemical change, this work will in turn have uncovered novel therapeutic targets. Future studies would involve rational drug design of therapeutic inhibitors of these enzymes that may lead to the treatment or prevention of breast cancer, ultimately leading to reductions in incidence and mortality from breast cancer.

PI Name: Robert Weinberg

Mechanism: Post Doctoral Fellowship

Institution: Whitehead Institute for Biomedical Research, MIT

Application Title: Influence of Cancer Stem Cell on Non-stem Cancer Cell: Growth, Metastasis and Stemness

Abstract:

Cancer stem cells (CSCs) are cancer cells that possess the ability to give rise to all cell types found in the cancer. Thus, these cells are tumorigenic, in contrast to other non-tumorigenic cancer cells (non-stem cancer cells, non-CSCs). CSCs can cause relapse and metastasis by giving rise to new tumors. Therefore, development of specific therapies targeted at CSCs is critical for improvement of cancer therapy. Our group has shown that invasive breast tumors can stimulate the growth of indolent breast tumors, which poorly grow on their own. This shows that some tumors can affect the behaviors of other tumor cells. Thus, we suspect that multipotent CSCs may affect the behaviors of non-CSCs. Furthermore, we have found that the characteristics of breast CSCs can be induced on non-CSCs. Thus, we suspect that CSCs may even induce non-CSCs to become CSCs. We will implant the non-CSCs with or without CSCs into the mice to see if non-CSCs behave differently under the presence of CSCs. If these hypotheses are proven to be true, we will further study how CSCs affect non-CSCs. Many current therapies cannot effectively kill CSCs and the disease can relapse from the minor portion of cancer cells. Since CSCs may induce non-CSCs to become CSCs, an efficient therapy of cancer should not only focus on eliminating the existing CSCs, which can unlimitedly grow, but also should block the route which is used by CSCs to convert non-CSCs to CSCs. If this hypothesis is true, how CSCs recruit non-CSCs into the CSC population will be a critical target for cancer therapy. To understand the media and signaling pathways which involve in the formation of CSCs can help designing an efficient therapy to prevent the formation and spreading of CSCs. The signaling pathways can be critical targets of breast cancer therapies to block the breast tumor growth, metastasis and even the formation of CSC.

PI Name: Roger Greenberg

Mechanism: Post Doctoral Fellowship

Institution: University of Pennsylvania School of Medicine

Application Title: BRCA1 Ubiquitin E3 Ligase Activity in DNA Damage Repair and Breast Cancer Suppression

Abstract:

Breast cancer is the most common malignancy in females in U.S with an incidence of nearly 13%. It is also the second leading cause of cancer-associated mortality. About 25-50% of inherited breast cancers stem from mutations in the BRCA1 and BRCA2 genes. BRCA1 and BRCA2 mutations account for a lifetime risk of 85% for breast cancer and 30-60% for ovarian cancer. BRCA1 is a tumor suppressor gene, whose loss of function results in tumorigenesis. One significant aspect from recent research on BRCA1 is the finding that BRCA1 is involved in DNA repair processes. DNA damage occurs due to environmental exposure to mutagenic chemicals or radiation as well as metabolic byproducts such as reactive oxygen species. Failure to repair DNA lesions leads to genomic alterations that favor cancer formation. Lending strong support to the hypothesis that BRCA1 DNA repair function is essential to prevent malignancy is the observation that BRCA1 interacts with other DNA repair proteins encoded by genes that are also mutated in inherited cancers. Identification of other critical components of this BRCA1 protein network is a productive means for discovering cancer susceptibility genes. Overwhelming clinical data implicate BRCA1 E3 ubiquitin ligase activity to be critical for tumor suppression. Nearly 20% of BRCA1 mutations disrupt BRCA1 E3 ligase function. E3 ubiquitin ligase is an enzyme that modifies a substrate protein by covalently attaching a protein of 76 amino acids known as ubiquitin. This modification, called ubiquitination, can promote substrate degradation, change substrate subcellular location, or alter substrate protein-protein interaction and function. Thus modifying a substrate with ubiquitin serves as a molecular tag to impart a specific activity to a protein substrate. My hypothesis is that BRCA1 E3 ubiquitin ligase activity plays an essential role in DNA double strand break repair and contributes to breast cancer suppression. BRCA1 dependent ubiquitination of its cognate substrates enables their active engagement in the checkpoint and homologous recombination activities required to execute the multifaceted tumor suppression functions of BRCA1. The research proposed here aims to determine the significance of BRCA1 E3 ubiquitin ligase activity in DNA repair and tumor suppression. Identification and characterization of the role of BRCA1 E3 substrates in the execution of BRCA1 dependent DNA damage response will be necessary to gain a sophisticated understanding of how BRCA1 controls genome integrity. In addition, upstream signaling pathways that regulate BRCA1 E3 ligase activity in response to DNA damage will be dissected in order to delineate pathways responsible for BRCA1 function. To directly address the role of BRCA1 E3 ligase activity in breast cancer suppression, mutant BRCA1 mouse models that selectively disrupting BRCA1 E3 ligase activity will be established. Thus an integrated biochemical and genetic investigation into BRCA1 E3 ligase function will be pursued in order to elucidate some of the basic mechanisms underlying control of genome integrity that are responsible for suppression of breast epithelial malignancy. In addition,

Pending Execution of Grant Agreements

these results will provide invaluable information to select new candidate genes for families with a high incidence of breast cancer without BRCA1 or BRCA2 mutations.

PI Name: Gregory Verdine

Mechanism: Post Doctoral Fellowship

Institution: Harvard University

Application Title: Targeting Wnt signaling pathway for breast cancer therapy

Abstract:

A high percentage of breast cancer patients under treatment will eventually develop aggressive recurrent or metastasized tumors that account for 50% of death caused by the disease. Recurrent or refractory breast cancer cells are the obstacles in breast cancer treatments since these cells usually gain drug resistance. One source of this cell type comes from studies of a small population of self-renewing, stem cell-like cells within tumors, which are called cancer stem cells. Cancer stem cells constitute a small minority of neoplastic cells within a tumor and are defined operationally by their ability to seed new tumors. For this reason, they have also been termed “tumor-initiating cells.” Recent research has suggested that cancer stem cells are refractory to current breast cancer treatments and highly metastatic, causing the failure of current treatment strategies. Several lines of evidence have indicated that the Wnt/beta-catenin signaling pathway plays a key role in cancer stem cell self-renewal, and inhibiting beta-catenin can turn off this effect. Unfortunately, beta-catenin is considered an “undruggable” target, lacking the deep hydrophobic pockets necessary for targeting by small molecule drugs and beyond the solely extracellular reach of protein therapeutics such as monoclonal antibodies. Therefore, a new class of drugs that can overcome this caveat will be highly valuable in clinics. Recent work in our laboratory has led to the discovery of a new class of targeting agents – hydrocarbon-stapled alpha-helical peptides – that enable the targeting of undruggable targets engaged in intracellular protein-protein interactions, including transcription factors. Building on our expertise, we plan to develop all-hydrocarbon cross-linked inhibitory peptides (stapled peptides) that will block beta-catenin signaling in breast cancer cells. Our chemical stapling strategy will efficiently stabilize peptide inhibitors while also conferring cell permeability essential to the targeting of intracellular proteins like beta-catenin. We are confident that this novel approach will yield potent inhibitors and could ultimately form the basis for development of a therapeutic agent for in cancer patients.

PI Name: John Carpten

Mechanism: Post Doctoral Fellowship

Institution: Translational Genomics Research Institute (TGen)

Application Title: Determining the significance of the AKT(E17K) mutation in human breast cancer

Abstract:

Breast cancer is the most common type of malignancy among women in the United States. Histological analysis reveals that the normal breast ducts, from which breast cancers often arise, is composed of an outer layer of myoepithelial (basal) cells and an inner layer of luminal epithelial cells. To investigate the influence of these normal cell types on the behavior of tumorigenic cell derivatives, a group recently developed a cell culture method that allows direct comparison of these different normal populations. This study showed that myoepithelial cells termed HME and the luminal epithelial cells termed BPE behave differently both in culture and in a mouse breast tumor model. When these cells are genetically engineered to become tumorigenic they form tumors in mice exhibiting major differences in histopathology, tumorigenicity, and metastatic behavior. The combination of acquired molecular changes also constitutes the clinical features of cancer. In this regard, a molecule known as a kinase and called Akt/PKB has emerged as the most commonly deregulated pathway in human breast cancer. AKT plays a pivotal role in many fundamental cell processes such as cell survival, cell proliferation, differentiation, blood vessel formation, metabolism, and cell death. We have recently identified a somatic mutation in the AKT1 gene, which results in a lysine to glutamic acid substitution (E17K) in human cancers. This mutation is constantly active by virtue of its intracellular localization, stimulation of other proteins, tumor formation in vitro, and induction of leukemia in mice. Overall the mutation is rare and is absent in some cancers including pancreatic, liver and B-cell derived leukemias. In breast cancer, reports indicate that the mutation frequency ranges from 1.4% to 8%, suggesting that the molecular and histological context of vulnerability may play an important role in the fate of acquiring the mutation. We now understand that the AKT1(E17K) is restricted to hormone-receptor positive breast cancers, is absent from all cell lines examined and is mutually exclusive with other important genes commonly implicated to breast cancer known as PTEN and P1K3CA. Some preliminary functional data from our lab, obtained by performing a cell growth assay in cells expressing wild-type (wt, normal) AKT1 or AKT1(E17K) (mutant) by viral-mediated gene delivery, suggests that E17K and to a lesser extent wt AKT1 has a marked inhibitory effect on cell proliferation in HME (myoepithelial) cells but a slight and equally proliferative advantage in BPE (luminal) cells. Interestingly, in the tumor-forming derivative of HME cells, known as HMLER, wt AKT1, and to a non-significant degree, E17K increased cell proliferation. On the other hand, E17K significantly inhibited cell growth whereas wt AKT1 had no effect in the tumor-forming BPE derived cells (BPLER). We confirmed that the mutation is activating in these cells by showing staining in the relevant intracellular compartment which differs from the localization of unstimulated wt AKT1. We also showed that the regulatory amino-acid residues, serine-476 and threonine-308, are hyper-phosphorylation in E17K but not in wt AKT1. Taken together, these initial studies suggest that the mammary epithelial cell of origin is an important determinant of AKT1 function. We suspect that wt AKT1 and E17K may initially equally assist in the

initiation of luminal-derived tumors but once a cell is transformed the mutation switches to prevent disease progression in the luminal cell setting. We suspect the role of the mutation in myoepithelial cells may serve to inhibit tumor initiation from the onset and as a result is selected against by tumor initiating cells, explaining the low frequency of the mutation. Therefore, we suspect the mutation may have a tumor inhibitory role and therefore if acquired may serve as a good prognostic indicator. Our current research study aims to understand the pathobiological role of a clinically relevant AKT1 mutation in human mammary epithelial cells by assessing the functional and molecular contexts as they relate to the histological normal cell of origin. The research design of this study is based upon three independent research aims designed to test our hypothesis. Our first aim is to analyze the regulation of oncogenic cell processes by AKT1(E17K) when compared to wt AKT1 and determine how these functions vary in myoepithelial and luminal epithelial cells. We will use a lentiviral gene transduction system to deliver wt or mutant AKT1 to assess cell viability, cell cycle, apoptosis, and anchorage independent growth. In addition, we will assess how the phosphorylation of well-known downstream AKT1 effectors differ for E17K. Our second goal is aimed at comparing the ability of E17K versus wt AKT1 to initiate tumor formation and contribute to tumor progression in an orthotopic mammary mouse model of breast cancer. The purpose of our last aim is set to discover novel E17K-relevant genes, signaling pathways or molecular signatures as they occur in myoepithelial and luminal epithelial cells. Global gene and protein expression profiling using Affymetrix cDNA microarrays and 2D-differential gel electrophoresis will be used to interrogate the transcriptome and proteome respectively of human mammary epithelial cells of interest. Elucidating the molecular and pathobiological significance of AKT1(E17K) will pave the way for future studies addressing the clinical and translational utility of a mutation in one of the most important genes implicated to human cancer.

PI Name: Hong-Ming Hu

Mechanism: Post Doctoral Fellowship

Institution: Providence Portland Medical Center

Application Title: Tumor-derived Autophagosomes as a Novel Vaccine in Breast Cancer

Abstract:

Breast cancer is the most common cancer and the second leading cause of cancer death in women in the United States. Conventional treatments including surgery, radiation, and chemotherapies, have improved survival, but they are generally associated with high toxicity, low tissue specificity, and are unable to cure patients with metastatic diseases. These drawbacks have led to an intense search for highly targeted immunotherapy that specifically destroys tumor cells with little or no side effects. Active immunotherapy is to boost the patient's own immune system by vaccination with tumor antigens to activate immune cells to fight against tumors. Antigen is a substance that prompts the generation of antibodies and can induce immune responses. In the past decade, a number of vaccines, such as peptides, proteins, whole tumor cells, and professional antigen presenting cells (APC) loaded with tumor antigens, has been tested in phase I and II breast cancer trials, but have met with failure. The unsatisfactory clinical outcomes are due to several factors including 1) the tumor antigens included in the vaccines were poor in activating immune cells 2) the antigens were not properly presented to immune cells and thus the immune cells were not activated 3) tumor escape due to antigen loss, and 4) suppression of the immune cells by suppressors. These limitations underscore the need to develop a novel, potent vaccine platform, which can circumvent the above-mentioned impediments. Here we propose that the tumor-derived autophagosome-based vaccine in combination with procedures to eliminate immune suppressors would be an effective vaccine for immunotherapy of breast cancer. Autophagosomes are double-membrane vesicles that sequester cytosolic proteins and damaged organelles when cells undergo autophagy, a "self-eating" cellular process. With special treatment of the tumor cells in vitro, a large number of cellular antigens and proteins that greatly facilitate activation of the immune system are sequestered in massive amounts in autophagosomes. We recently reported that autophagy of tumor cells is essential for presentation of tumor antigens and activation of immune cells. More importantly, we showed that vaccination with tumor-derived autophagosomes was effective in treating melanoma and Lewis lung carcinoma in mice. Moreover, autophagosomes from sarcoma cells protected against another cell line of sarcoma that was distinctive in antigen expression, suggesting that autophagosomes carry shared tumor antigens and may protect against a related tumor from another patient. This observation provides the possibility of making an off-the-shelf breast cancer vaccine from established cell lines. To further enhance immune responses, adjuvant will be used in the vaccine. To block immune suppression, anti-c-kit antibody will be included. In this application, we propose to test the efficacy of tumor-derived autophagosome-based vaccines in treating the highly metastatic 4T1 breast cancer in BALB/c mice. We hypothesize that vaccination with tumor-derived autophagosomes combined with the adjuvant and the inhibitor of the immune suppressors mediates regression of the same tumor by eliciting robust immune responses, and also provides protection against other related tumors through shared antigens. To test our hypothesis, our specific aims are as follows: 1) To determine the efficacy of 4T1 tumor-

Pending Execution of Grant Agreements

derived autophagosome-based vaccine in treatment of established 4T1 breast tumor. 2) To examine the mechanisms on how the immune cells are activated by vaccination and how they eradicate tumors. 3) To evaluate the ability of the vaccine to protect against a different breast tumor. Study Design: Multiple vaccinations will be administered subcutaneously to mice with transplanted tumors. Tumor growth and overall survival will be measured. Immune cell responses, tumor infiltrating immune cells, and frequency of immune suppressor cells will be assayed in the proposed study. The expected outcomes include 1) the autophagosome vaccine alone will lead to regression of the same tumor in the mouse model; the combined immunotherapy will improve the anti-tumor efficacy. 2) The combination therapy will induce robust immune responses and reduce accumulation of immune suppressor cells. 3) autophagosome vaccine will provide partial protection against a different breast tumor. The proposal will provide the proof-of-principle for a novel clinical tool that can be translated into clinical trials in patients with breast cancer. Delineation of the mechanism of immune protection will contribute greatly to our understanding on how the immune system can be positively modulated by active immunization to fight against cancer. Any benefit of the proposed approaches will be exploited to develop autophagosome-based vaccines for treatment of breast cancer patients.

PI Name: Michael Mancini

Mechanism: Post Doctoral Fellowship

Institution: Baylor College of Medicine

Application Title: Application of high-content, imaging-based assays to the study of environmental and dietary estrogens in breast cancer

Abstract:

Breast cancer is the most common form and 2nd leading cause of cancer-related death in women. While normal levels of the hormone estrogen are critical to health, it is accepted that prolonged or over exposure to estrogen can cause breast cancer. This can result from natural influences such as aging but pharmaceutical estrogens including hormone replacement therapies also increase the risk. It is not surprising then, that massive concerns were raised when several compounds in our environment and diet were shown to behave like estrogen. Some are now being linked to causing a predisposition to breast cancer. However, partly due to conflicting data from human and animal studies, many such compounds remain in general use. It is possible that limiting exposure to estrogenic chemicals could significantly reduce preventable breast cancers. Additionally, dietary compounds, e.g. soy, that are reported to block estrogen have the potential to be chemopreventive but again human and animal studies remain inconclusive. It is therefore imperative to develop new and faster tools to determine the potential of these compounds to be harmful or beneficial so we can limit our exposure or use them to develop new chemopreventive strategies. In this proposal I will develop and use image-based technology to generate measurements that characterize the total response of a cell to a panel of environmental and dietary compounds in order to to define their estrogenic, carcinogenic and chemopreventive potential based similarities and differences to known carcinogen estradiol versus known chemopreventative tamoxifen. Approximately 75% of breast cancers express estrogen receptor (ER). Not all are responsive to treatment with the selective estrogen receptor modifiers (SERMs) tamoxifen and raloxifene. In some cases ER positive tumors are tamoxifen-resistant or more commonly the tumors develop resistance. This results in tamoxifen activating ER and promoting cancer growth instead of being chemopreventive. This resistance has been linked to over-expression of the human epidermal growth factor receptor (Her) 2. The second aim of this proposal is to use the image-based assay I have developed to investigate the role of Her2 expression in environmental compound activity with the hope of finding mechanisms by which tamoxifen-resistant cancers could be targeted with chemotherapies.

PI Name: Ratna Vadlamudi

Mechanism: Post Doctoral Fellowship

Institution: University of Texas Health Science Center at San Antonio

Application Title: PELP1: A novel therapeutic target for breast cancer metastasis

Abstract:

Despite these advances in breast cancer therapy, the prophecies made by the scientists to make breast cancer curable by the 21st century appears to be wrong. Endocrine therapy has also been shown to have a positive effect on the treatment of advanced metastatic disease. Despite these positive effects, initial or acquired resistance to endocrine therapies frequently occurs, with tumors recurring as metastasis (often as ER-ve), which is the leading cause of death from breast cancer. Although the long term goal still remains to make this life threatening disease curable one day, our understanding to do so is yet not comprehensive enough. The short term goal is thus to turn the deadly disease into a chronic one: to elongate the survival time of the cancer patients with a maximum of life quality. In order to reach this short term goal, two features of cancer have to be taken into account. First, the growth of the tumor must be contained. Second, the spreading of the tumor and the development of (micro-) metastases must be inhibited. Thus controlling growth and metastasis development are the crucial parameters to improve patient quality of life. To appreciate the mechanisms by which breast cancers develop into metastasis, it is necessary to understand molecular mechanism(s) involved in metastasis. During the past 20 years, studies have extensively been focused on the role of two nuclear receptors ER and PR. Even though ER and PR explain the biology of ER+ve tumors, it remain unknown as to what drives ER-ve metastatic tumors. Recent advances in detection technologies suggest that other nuclear receptors and their interacting coregulators may play a role in the growth, and metastasis of ER-ve tumors. There is critical need to identify novel targets that can be therapeutically used to curb the progression of breast cancer metastasis. The rationale for the study emerges from recent studies in our laboratory that (1) PELP1 (Proline, Glutamic acid, Leucine rich Protein 1) functions as a potential proto-oncogene, (2) PELP1 interacts with a wide variety of nuclear receptors and participates in genomic and nongenomic actions of nuclear receptors (3) PELP1 expression is deregulated in metastatic tumors and (4) high PELP1 expression is maintained in node positive and metastatic ER-ve breast tumors. The central hypothesis is that during progression from tumorigenesis to invasion, PELP1 trigger signals in tumor cells that activate both genomic and nongenotrophic signaling pathways leading to enhanced cell migratory functions and metastasis. The objective of this proposal is to examine whether proto-oncogene PELP1 contributes to metastatic potential of ER -ve breast cancer cells and to test whether blocking of PELP1 signaling axis will have therapeutic effect. The hypothesis will be tested using two specific aims; Aim 1. To establish the significance and mechanism of PELP1 actions in metastasis. Aim 2. To determine the therapeutic potential of blocking PELP1 axis in metastasis. To accomplish these goals, in Aim 1, we will generate novel invasive model cells with or without functional PELP1 axis by stably transfecting control of PELP1 specific shRNA. We will use these model cells in different assays (migration, invasion and MMP) to decipher the role of PELP1 in breast cancer cell migration and invasion. We will use pathway specific microarrays to identify PELP1 target genes in metastasis. In Aim 2, we will test the significance of PELP1 in

Pending Execution of Grant Agreements

in vivo metastasis using syngenic as well as nude mice models.. We will also generate targeted PELP1siRNA_Nanoparticles to ascertain the significance of PELP1 axis in the cell migration and test the effect of blocking PELP1 expression in vivo to prevent or delay metastasis. Collectively results obtained from both these aims, will determine whether PELP1 is a rate limiting factor in the initiation and progression of ER-ve breast cancer metastasis. The proposed research will establish the significance of PELP1 actions in cell motility/metastasis and also determine the therapeutic potential of blocking PELP1 actions in addition to current therapies to combat metastasis. At the completion of this project it is our expectation that we will have identified a novel pathway that contribute to metastasis potential of ER-ve tumor cells and test the ability of PELP1 nanoparticles as a drug to block ER-ve metastasis. This is innovative because the proposed specific aims and novel methodology will lead to the identification of signaling pathways that connect PELP1 with the cytoskeleton, and thus may provide novel targets for combination therapies to treat metastatic and advanced breast tumor. Understanding how NR coregulators play a role in metastasis will be useful in maximizing treatment opportunities for metastatic breast cancer.

PI Name: Bert O'Malley

Mechanism: Post Doctoral Fellowship

Institution: Baylor College of Medicine

Application Title: Defining the Role of Post-Translational Modifications in SRC-3-mediated Repression of mRNA Translation

Abstract:

The most common, malignant tumor found in women living in North America is breast cancer. In response to a public outcry for research to be conducted that will help us understand how breast cancer develops, scientists have found that genes in normal breast cells are damaged and that this damage can turn a normal breast cell into a cancerous breast cell. While changes within breast cells clearly promote the development and metastasis of breast cancer, an exciting new field of research focused on the tumor environment has emerged. Recent data has shown that when oncogenic events are initiated within breast cells, the immune system is activated. Activation of the immune system triggers the influx of a variety of immune cells into the breast tissue environment. At first these immune cells destroy pre-cancerous breast cells, helping to stop breast cancer from developing. However, if the breast cancer cells survive, like many of them do, over time, the immune cells develop properties that actually start to promote the progression of breast cancer. One such property is the release of small signaling proteins called cytokines from a type of immune cell called a macrophage into the breast. These inflammatory cytokines enhance tumor metastasis. Clearly, understanding how cytokines are regulated is of critical importance towards understanding how breast cancer develops and progresses to a more aggressive state. There is relatively little scientific data focused on understanding how cytokines are produced, and more importantly, how they can be inhibited. However, our laboratory has recently discovered that the protein steroid receptor coactivator 3 (SRC-3) inhibits the production of cytokines from macrophages. Others have shown that SRC-3 is involved in many other biological processes besides inhibiting cytokines, some of these processes could help tumor growth and others could inhibit tumor growth. So, we wonder how SRC-3 can participate in so many different biological functions and how we could direct SRC-3 so that it is concentrated on suppressing inflammatory cytokines that promote breast cancer progression. One way that the cell can direct SRC-3 in one direction versus another is through the addition of small chemical moieties to the SRC-3 protein called post-translational modifications (PTMs). There are many different types of PTMs that can act as a code, channeling SRC-3 protein towards participating in one activity versus another. I hypothesize that specific PTMs provide a code, directing SRC-3 towards inhibiting the production of tumor-promoting cytokines. In this proposed study, I will perform experiments that will identify which PTMs are necessary for SRC-3 to inhibit the production of inflammatory cytokines. Additionally, I will determine how these PTMs direct SRC-3 to inhibit cytokine production. Finally, I will test how SRC-3 molecules that are 'coded' with PTMs in macrophage cells impact breast cancer. We will test if the SRC-3 protein produced in macrophages and containing these specific PTMs decreases tumor-associated properties of breast cancer cells that help the tumor to metastasize. These properties include the ability of the breast cancer cells to migrate and potentially invade other tissues. This proposed study will provide unique pieces of information as to how SRC-3 is directed to inhibit tumor-

Pending Execution of Grant Agreements

promoting cytokines in macrophages, and it will provide data as to how SRC-3 that is synthesized in macrophages affects breast cancer progression. From this information, therapies could be developed to program SRC-3 so that it only has PTMs that direct it towards activities that inhibit breast cancer progression. This would be a brand-new approach to treatment of breast cancer, as other research has only focused on how SRC-3 molecules act within breast cancer cells and not within macrophages. Such therapies that inhibit tumor metastasis would lead to a decrease in mortality from this disease.

PI Name: Christopher Turner

Mechanism: Post Doctoral Fellowship

Institution: State University of New York, Upstate Medical University

Application Title: Paxillin regulates matrix metalloproteinase trafficking/recycling and breast cancer cell invasion in three-dimensional microenvironments

Abstract:

Breast cancer affects 1 in 8 women, with an estimated 178,000 new cases of invasive breast cancer diagnosed in America in 2007 (American Cancer Society, Surveillance Research, 2007). Alarming, breast cancer prevalence has shown an increase over the past four decades. The single worst prognostic factor dictating patient long-term survival is the occurrence of metastasis. The process of metastasis involves the movement, also known as invasion, of tumor cells from the site of the primary tumor growth, through the surrounding tissue, to the lymph system or blood vessels and subsequent colonization and growth in remote tissues, such as bones and lung. The environment surrounding the primary tumor is called the stroma and is made up of a dense fibrous network of proteins called the extracellular matrix (ECM), which includes proteins such as collagens. Cancer cells utilize two mechanisms for migrating through this dense meshwork of proteins. Firstly, cells are able to degrade the ECM ahead of them, by secreting proteins called matrix metalloproteinases (MMPs) to dig a tunnel through which they and other cancer cells can drag themselves through to gain access to the lymph or blood system. This is known as mesenchymal migration/invasion and requires the cell to interact with the proteins of the ECM to pull itself through the hole created by MMPs. The second type of cancer cell movement is known as amoeboid migration/invasion, which does not require MMPs or the cell to interact with the ECM. During amoeboid migration cells squeeze through tiny gaps in the stroma to reach their target. Indeed, the ability of cancer cells to switch between these two types of migration/invasion has made MMP-targeted drugs largely ineffective in treating metastases. Cells attach to the ECM through proteins on the cell surface known as integrins, the “molecular Velcro” that act like feet, allowing the cell to walk and to invade tissue. Integrins cluster together and form structures known as adhesion contacts, which recruit other proteins inside the cell allowing the cell to regulate its attachment to the stroma and allow it to pull itself through the ECM. One of the proteins recruited to adhesion contacts is paxillin, a cellular protein able to interact with numerous other proteins, acting as a type of scaffold to coordinate their function. Cells in which we have blocked paxillin production were unable to invade or switch between the two types of invasion. Thus, we hypothesize that paxillin is a key regulator of breast cancer metastasis through controlling the balance between the two types of invasion (mesenchymal and amoeboid) potentially through altering the function of both MMPs and integrins. Through the use of breast cancer cells and models for tumor invasion we will identify the mechanism, by which paxillin controls the invasion of breast tumor cells. We have also shown that upon removal of paxillin, breast cancer cells have altered MMP function and secretion. These cells also display enhanced secretion of structures, containing MMPs, which have previously been shown to prevent tumor growth and promote tumor attack by the body’s immune defenses. In this study we will also dissect the mechanism by which paxillin is able to regulate MMP function and how it normally suppresses the release of these tumor-preventing structures.

Pending Execution of Grant Agreements

This project will identify a new therapeutic target pathway for drug development, which will be able to inhibit breast cancer metastasis through preventing tumor cell invasion. Drugs targeting paxillin or its functions in the cell may also promote primary tumor death, while also inhibiting metastasis to secondary sites. In combination with early detection through regular screening, therapeutics targeted at paxillin function have the potential to greatly decrease metastatic breast cancer incidence by providing a unique opportunity to attack numerous aspects of the disease and therefore enhance patient prognosis and survival.

PI Name: Josh Lauring

Mechanism: Post Doctoral Fellowship

Institution: Johns Hopkins University, Kimmel Cancer Center

Application Title: Investigating the mechanism of regulation of HOXA5 and consequences of its loss in breast cancer

Abstract:

Cancer is often considered a disease of some normal step in development which went terribly wrong. These abnormal cells linger and collect more and more “hits” and eventually become cancers. In fact the recent stem cell or progenitor cell hypothesis of cancer suggests the same thing. We study HOX proteins in breast cancer. These are master regulators of expression of key genes during embryonic development and are responsible for proper placement of body structure in our body- the head where the head is, and the feet where the feet ought to be. A few years ago, members of our breast cancer program showed that the expression of HOXA5 is lost in >60% breast cancer cell lines and primary tumors. Restoring the missing HOXA5 protein in cancer cells resulted in their death, suggesting that HOXA5 may be a tumor suppressor gene in breast cells. So what controls HOXA5 expression in cells and how does HOXA5 play its role in breast cancer development? Recent studies have discovered small RNA molecules that do not make protein, and are called noncoding RNAs. These are located right next to each HOX gene, and when the ncRNA level goes up, the HOX protein level goes up too and vice versa. So do ncRNAs control the expression of HOXA5? There is evidence to point out that ncRNA may play an important role in HOXA5 expression and its loss in breast cancer. In this proposal, we will combine the information already existing in breast cancer databases and HOX cluster microarray analysis 1) to identify ncRNAs candidates important for the regulation of HOXA5, and 2) to study the function of these ncRNAs in breast cancer cells. Secondly, to study HOXA5 function and the consequences of its loss in a more natural setting and using a unique expertise in my mentor’s lab, we have created a new mammary epithelial cell model. Here, the HOXA5 gene is partially (1 copy deleted) or completely (both copies deleted) disabled in MCF10A, a normal, diploid breast epithelial cell line using recombinant DNA technology. Using these HOXA5-null cells I would like to study how HOXA5 loss can promote malignant transformation of MCF10A cells. Our early data suggests that HOXA5 may control the expression of a very important growth factor receptor, epidermal growth factor receptor or EGFR, which contributes to breast cancer in many ways. Using a variety of molecular and cell biology techniques, 1) I will determine if HOXA5 is a direct transcriptional regulator of EGFR, and compare the changes of EGFR signaling between wild type MCF10A, HOXA5 knockout cells MCF10A (+/-), and MCF10A (-/-) treated with different concentrations of EGF, and 2) extend these studies to other breast cell lines models. 3) Is HOXA5 truly a tumor suppressor gene? Can it transform cells all by itself or does it need a combination of mutated genes to do so? To determine whether loss of HOXA5 is able to promote transformation of MCF10A cells alone or in combination with mutant H-ras or PIK3CA oncogene tests such as growth in low nutrient medium and growth in soft agar or as tumors in immunodeficient mice will be performed following transfection of the cells with one or both genes. The work proposed here has the potential to provide insights into the role of HOXA5 in mammary carcinogenesis. As we know that EGF and EGFR have been at the

Pending Execution of Grant Agreements

forefront of cancer therapeutics. Targeting of ncRNA might provide an important therapeutic strategy for cancer treatment. We hope that our study will provide valuable information to identify molecular as new diagnostic markers or therapeutic target for breast cancer and to provide me with a solid understanding of breast cancer and the best training to conduct research and to ensure my future success as an independent researcher in breast cancer.

PI Name: Xiaojing Ma

Mechanism: Post Doctoral Fellowship

Institutions: Cornell University, Weill Medical College

Application Title: Role and mechanism of chemokine RANTES in immunity against breast cancer

Abstract:

Host immune competence is essential for controlling the growth of invasive tumors. Tumor-specific immunity is a highly desired outcome of any successful anti-tumor therapy. Many types of tumors, including breast cancers, are able to evolve mechanisms that allow them to escape detection and attack by the immune system. CCL5 is a chemokine produced by a variety of human cells in response to stress. It plays an essential role in inflammation by recruiting T cells, macrophages, and eosinophils to inflammatory sites. The expression of CCL5 has been linked to cancer progression in a number of studies. Tumor-derived CCL5 can help tumors form new blood vessels (angiogenesis), inhibit the T cell response to tumor cells, and enhance the growth of mammary carcinoma (breast cancer). In humans, CCL5 expression correlates with cancer stage. There is a high incidence of CCL5 expression in tissue sections of breast carcinomas, while it is rarely detected in normal duct epithelial cells or in epithelial cells of benign breast lumps. Thus, CCL5 expression may be an early indication of an otherwise undetectable malignant process. The murine 4T1 mammary carcinoma cell line is a breast cancer model that closely resembles human breast cancer in primary tumor growth characteristics and metastatic properties. 4T1 mammary tumors are highly aggressive, metastatic and poorly immunogenic. Mice that are genetically deficient in CCL5 (designated CCL5-null), however, are highly resistant to 4T1 tumor growth and to tumor-induced death compared to wild type controls. In addition, the tumors in CCL5-null mice produced few lung metastases after 60 days. Those CCL5-null mice that had rapid tumor regression rejected rechallenge with 4T1 tumor cells, indicating establishment of protective immunity. We hypothesize that CCL5 plays a critical role in the growth and metastasis of mammary tumors, and that blocking CCL5 in tumor-bearing hosts will have a strong therapeutic impact without overt side effects. We plan to explore the following therapeutic approaches to blocking CCL5 expression/function in vivo in our animal model to determine their impact on 4T1 breast cancer growth and metastasis: 1. Investigate the immunological mechanisms by which CCL5 promotes mammary tumor growth. 2. To block the spontaneously high levels of CCL5 in tumor-bearing mice using specific monoclonal antibodies. 3. Develop a highly robust and large-scale test to identify small molecule inhibitors of mouse and human CCL5 for potential pre-clinical and clinical applications, respectively. CCL5 represents a desirable and accessible target for breast cancer therapy because of its relevance to human breast cancer progression and its apparent dispensability in development and general immunity. This investigation will likely uncover the mechanisms whereby CCL5 promotes mammary tumor growth. We will also likely identify and characterize small molecule inhibitors of CCL5 for therapeutic testing in the animal model. These studies will facilitate the future design of drugs for the treatment of breast cancer in human clinical studies, as an extension of this study.

PI Name: Celeste Nelson

Mechanism: Post Doctoral Fellowship

Institutions: Princeton University

Application Title: Effect of Matrix Compliance and Mechanical Tension on Fibrosis of the Breast

Abstract:

During normal wound healing processes specialized cells called myofibroblasts exert force on their surrounding environment and synthesize large quantities of proteins to assist in tissue repair. Typically, when repair is complete the proteins deposited to aid in healing are degraded and myofibroblasts at the site undergo programmed cell death. Deregulation and inappropriate activation of myofibroblasts can lead to stiffening of the tissue and loss of tissue function resulting in a condition called tissue fibrosis. Increasing evidence suggests that the risk for a variety of cancers, including breast cancer, increases with the presence of fibrotic lesions. Our ultimate goal is to understand how the mechanical properties of the protein matrix surrounding cells affect and are affected by the development of fibrosis. We hypothesize that matrices with stiffnesses near to that of normal tissue are protective against inappropriate activation of myofibroblasts. Specifically, through the use of both two- and three-dimensional tissue culture platforms in which the stiffness of the protein matrix can be varied over a range encompassing that of the normal mammary gland and the average breast tumor, we will investigate how the mechanical properties of the cell environment affect the development of myofibroblasts from normal mammary cells. The roles of various proteins within the cells that react to the mechanical properties of the surrounding matrix and influence cellular behavior will be probed in an effort to identify molecular mechanisms necessary for this process. Lastly, we will explore how the development of myofibroblasts from normal mammary cells changes and remodels the properties of the surrounding matrix. Collectively, the results will provide the framework for a model of mammary tissue disruption and fibrosis, and will aid in the discovery and evaluation of therapeutic strategies directed toward treatment and prevention of fibrosis and tumor progression.

PI Name: Luisa Franzini

Mechanism: Post-Baccalaureate Training in Disparities Research

Institution: University of Texas at Health Science Center at Houston

Application Title: A transdisciplinary training programs for public health researchers and practitioners wanting to impact breast cancer disparities

Abstract:

1. Study hypothesis Susan J. Komen for the Cure is dedicated to curing breast cancer at every stage and to ensure quality care for all. Unfortunately, wide disparities persist in breast cancer. A disproportionate number of breast cancer deaths occur in women of color (African American, Latino and other minorities women) and in women with fewer socioeconomic resources, for example women who are poor. The causes for these disparities could be that women of color and poor women are more likely to be diagnosed with more advanced breast cancer, have more aggressive tumors, and are less healthy. It is also possible that disadvantaged women receive worse quality treatment. Additionally, other factors can cause disparities in breast cancer survival: disadvantaged women have less access to care, they are more likely to be uninsured, they may have behavior and lifestyle that adversely affect their health, and may have a worse quality relationship with their physician. To eliminate breast cancer disparities, research is needed on the relationship between these factors and breast cancer screening, treatment, and survival in order to identify targets for interventions and policies. We need to train a new cadre of innovative and highly skilled researchers, preferably from populations who are disproportionately affected by breast cancer, who are committed to the elimination of breast cancer disparities. The objective of the training program we propose is to encourage the brightest doctoral students at the University of Texas School of Public Health (UTSPH) to pursue careers as researchers or public health practitioners focusing on breast cancer disparities. The hypothesis is that these students will pursue research or practice careers in breast cancer disparities and will reduce disparities through their research and practice.

2. How the training program advances the understanding of disparities and leads to a reduction in disparities The program aims to provide trainees (the students in the training program) with the necessary training and skills needed to successfully reduce breast cancer disparities by offering a rigorous specialized training program that includes formal coursework and mentored research and practice experiences. Specifically, we want to: 1. Recruit excellent students; 2. Provide excellent training; 3. Ensure that graduating trainees pursue a career in breast cancer disparities. The proposed training program will focus on critical problems that contribute to disparities in breast cancer. In particular, the program will focus the training on providing the necessary skills to address breast cancer disparities by providing a rigorous specialized curriculum in different disciplines, including epidemiology, biostatistics, health economics/outcomes research, policy, and social and behavioral sciences. Trainees in the specialized curriculum will participate, with mentoring faculty, in developing individual education and research plans. They will attend courses that cover skills and knowledge that are important in disparities research. They will participate in research and practice projects by choosing from several ongoing research or practice projects directly relevant to breast cancer disparities at UTSPH, MD Anderson Cancer Center, or in at-risk communities. UTSPH is a good environment for this training program because it has excellent research, computer, and

Pending Execution of Grant Agreements

library facilities. It also has excellent faculty who are interested in reducing breast cancer disparities and currently do state of the art research. It also has many students from minority and poor backgrounds, who could be trained to reduce breast cancer disparities in their communities. The knowledge obtained through the training program will provide them with the skills necessary to develop and implement public health interventions and policies that contribute to the reduction of breast cancer disparities. We expect all trainees graduating from the training program to engage in a research or practice career that contributes meaningfully to reductions in breast cancer disparities.

3. Importance of this program to patients with breast cancer This program is important to patients with breast cancer because many patients with breast cancer are women of color and women with fewer socioeconomic resources. They tend to be diagnosed with more advanced breast cancer, to be sicker, and to have more problems obtaining good quality care. Because of these and other reasons, they are more likely to die of breast cancer than more advantaged breast cancer patients. It is very important for the disadvantaged patients that researchers understand exactly why they have worse survival than more advantaged patients and what can be done to eliminate these differences in breast cancer survival. That is why we need to train researchers and practitioners, preferably from disadvantaged backgrounds, who are committed to eliminating breast cancer disparities in their communities and among all breast cancer patients. We plan to recruit excellent students into our training program and give them excellent training with the best researchers at the UTSPH and MDACC (the top cancer center in the U.S.). Our goal is that students who graduate from the program focus their careers on the elimination breast cancer disparities so that breast cancer patients of color and of disadvantaged background get the same quality treatment and survival as more advantaged patients.

Pending Execution of Grant Agreements

PI Name: Ann Klassen

Mechanism: Post-Baccalaureate Training in Disparities Research

Institution: Johns Hopkins University, Bloomberg School of Public Health

Application Title: Johns Hopkins Bloomberg School of Public Health Training Program in Breast Cancer Disparities Research

Abstract:

This application proposes the creation of a training program in breast cancer disparities research, within the existing Masters in Health Sciences and Doctor of Philosophy degrees programs in the Department of Health, Behavior and Society at the Johns Hopkins Bloomberg School of Public Health. The goals of this program will be to draw on the existing strengths of the School of Public Health, including the NIH-funded Hopkins Center for Health Disparities Solutions, as well as the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, and to provide the intellectual environment and resources to develop scholars in breast cancer disparities, specifically among talented students who may lack resources to fulfill their intellectual potential in this important area.

The program will offer tuition and stipend support for three post-baccalaureate students pursuing either MHS or PhD training. Priority will be given to recruiting applicants from several key backgrounds. The first is students who are from racial, ethnic and social groups within the US who have been traditionally underrepresented in post-baccalaureate training, including African Americans, Native Americans, Hispanic Americans, and South East Asian Americans, as well as persons representing the first generation of their family to attend college. The second priority group is students from countries outside the US where breast cancer burden is substantial and increasing, especially societies lacking the infrastructure and resources to offer research training. The third priority population is students who are themselves cancer survivors, and thus will become survivor-scholars in the field of cancer research. All three of these populations offer the potential to expand and enhance our scientific perspectives and expertise to address breast cancer disparities, but all three groups face significant barriers to obtaining the research skills and professional development necessary to make their potential scientific impact. Thus we have chosen to focus primarily on attracting and supporting these students.

The program will support students as they build both classroom based and experiential skills. Core coursework at both the doctoral and masters level will include: research design, analytical and quantitative methods, environmental science, nutrition, epidemiology, cancer prevention and control, and theories of health education, health communication, and social influences on health. In addition, the program will include special emphasis on understanding and intervening on breast cancer disparities, including journal clubs and research practicum in focused areas: genetic counseling and communication, health literacy and cultural competence, media analysis, poverty and social policies, cross-cultural and international issues in breast cancer, and strategies for cancer prevention, including tobacco control, nutrition, energy balance, and community-based participatory research. Each student will also be supported in conducting a practicum and manuscript (MHS) or doctoral research dissertation (PhD) in their chosen area of focus. As part of this preparation, they will have mentored research opportunities with the faculty investigators, all of whom are well-established researchers in health disparities. Additionally, the program will

Pending Execution of Grant Agreements

offer the students support to participate in scientific meetings in cancer disparities, with the goal of presenting their own work during their training. As needed, trainees will be offered support to strengthen any weaknesses in quantitative or scientific writing skills. In addition to the core program faculty, the training program participants will benefit from guidance from a strong network of clinical, basic science, and public health faculty at Johns Hopkins conducting research in health disparities and breast cancer. The School's strong contributions to local and State cancer control efforts in Baltimore and throughout the region, and proximity to the National Cancer Institute at NIH, will offer additional opportunities. Finally, this specific program will be partnered with our existing cross-departmental training program in cancer epidemiology, prevention and control, which has a 25 year history of successfully developing public health students into outstanding cancer researchers.

Pending Execution of Grant Agreements

PI Name: Kathy Baumgartner

Mechanism: Post-Baccalaureate Training in Disparities Research

Institution: University of Louisville

Application Title: University of Louisville: Susan G. Komen Breast Cancer Disparities Epidemiology Research Training Program

Abstract:

The objective of the Susan G. Komen Breast Cancer Disparities Epidemiology Research Training Program at the University of Louisville will be to train pre-doctoral students towards the knowledge and skills necessary to study the epidemiology of socioeconomic, geographic and racial/ethnic disparities in breast cancer risk and prognosis, integrating genetic, environmental, and socio-cultural perspectives. The training program will recruit students from the newly established PhD in Public Health Sciences Concentration in Epidemiology in the University of Louisville, School of Public Health and Information Sciences. Trainees will receive a focused program of didactic coursework, mentored readings, and hands-on research experience in one or more on-going epidemiologic breast cancer studies. The student's research project will result in a doctoral dissertation and one or more published papers.

Recent studies indicate that, while African American and Hispanic women are less likely to get breast cancer than non-Hispanic White women, they are more likely to develop a form that is resistant to therapy and to have a worse prognosis. It is not clear why they are more likely to get this form of breast cancer. Other studies indicate that African American and Hispanic women have poor prognosis because of lower levels of mammography screening, delayed diagnosis, and problems with access to care associated with lower socioeconomic status. It is not clear how these factors interact with biological factors that influence the type of breast cancer. The Co-Principal Investigators for the Komen Breast Cancer Disparities Epidemiology Research Training Program are conducting studies directly addressing the relative importance and interaction of these biological, environmental, economic, and cultural factors that alter risk and prognosis for breast cancer across racial/ethnic and socioeconomic groups. Komen Trainees will work with the Co-PIs to develop and test hypotheses that are appropriate for further evaluation of breast cancer disparities. The program will produce new epidemiologists with the necessary orientation and skills to conduct translational research leading to major reductions in breast cancer incidence and recurrence and further improvements in survival and quality of life.

Pending Execution of Grant Agreements

PI Name: Maria Elena Martinez

Mechanism: Post-Baccalaureate Training in Disparities Research

Institution: University of Arizona at Tucson

Application Title: A Post-Baccalaureate Training Program to Address Breast Cancer Disparities in Mexican/Mexican American Women

Abstract:

Breast cancer disparities in Hispanic women are due to several factors related to the factors in which the disease presents. Hispanic women are diagnosed at earlier age and with more aggressive tumors that results in lower probability of survival. Also, little information exists on the risk factors for breast cancer in Hispanics, their susceptibility, and if these factors are related to the different types of breast cancer that occurs in this population. All these features combined result in a significant disparity related to lack of knowledge about the disease, which results in a poor understanding of how to manage and treat breast cancer and thus poor outcome from the disease. The main scientific objective of the research aspect of the training grant is to recognize the presentation of breast tumors in women of Mexican descent who live in the U.S. and in those living in Mexico and to identify the important factors that influence this disease presentation and outcome (both genetic and environmental). Trainees will have the opportunity to be involved in assessing whether or not any observed differences in tumor markers are related to factors that are the result of a lifestyle that is more common in the U.S. or if the differences between the groups are the result of specific exposures or if they are more genetically related. The proposed training plan involves an approach that includes several branches of science that include Epidemiology, Genetics, Pathology, Molecular Biology and Disparities research. Our hypothesis is that the distribution of breast cancer types differs between women in Mexico and Mexican American women in the U.S. and that these differences are related to reproductive factors (such as age at first period, age at first birth, number of children, etc.) and lifestyle factors (physical inactivity, obesity, alcohol consumption, etc.). We further hypothesize that the differences we discover in the different tumor types between the two countries are related to a mix or interaction between genetic and lifestyle. To test our hypotheses, we are addressing the following research objectives: 1) to compare the pattern of tumor markers that determine prognosis and/or predict clinical importance (ER, PR, HER-2/neu, Ki67) between women in Mexico and Mexican women in the U.S.; 2) to compare tumor marker patterns that are associated with the different types of tumors between women in Mexico and Mexican women in the U.S.; 3) to determine whether differences in the markers are stronger in younger women compared to older women and whether these differences are the result of lifestyle factors related with a U.S. lifestyle (for example having no children, older age at first birth, obesity, being physically inactive, etc.); 4) to determine whether certain genetic factors together with lifestyle factors interact to cause certain types of breast cancer.

The training in breast cancer disparities will be based on the existing resources in the Ella Binational Breast Cancer Study, a study that is funded by the National Cancer Institute and the Avon Foundation to better understand breast cancer in women of Mexican descent. The Ella Study represents an invaluable and unique resource to conduct training in breast cancer disparities given that few studies like this one exist in or outside the U.S. The

Pending Execution of Grant Agreements

primary training objective of the program is to provide a structured program that is matched specifically with each trainee to empower him/her with the necessary skills needed to address and eliminate breast cancer health disparities in Hispanics. To accomplish this objective, the following training aims have been identified: 1) to identify and recruit three outstanding applicants from different scientific backgrounds who demonstrate an interest in breast cancer disparities research; 2) to provide the foundation for trainees to become familiar with the area of breast cancer across different racial/ethnic groups and the disparities applicable to the Hispanic populations; 3) to develop research, analytic, and scientific skills necessary to effectively conduct research in breast cancer disparities and translate these findings into public health practice; and, 4) to provide opportunities to all trainees to work across different scientific disciplines by participating in a curriculum-based program focused on addressing breast cancer disparities in Hispanic women.

The lack of information on characteristics of invasive breast cancers in Hispanic women highlights the high significance of our study. The U.S. significance of training is further supported by the rapidly increasing number of people of Hispanic origin in the U.S. Based on what has been learned in non-Hispanic white women, it is clear that a better understanding of breast cancer, including the specific types of breast tumors, arising in Hispanic women is needed and will improve treatment and decrease deaths in this population. An important step towards achieving this goal is the training of investigators ready to address the breast cancer burden and its related disparity in Hispanic women.