

Susan G. Komen for the Cure Research Grants – Fiscal Year 2010

This slate of research grants was approved by Komen’s national board of directors on March 18, 2010. These grants will be funded upon the execution of grant agreements between Komen and the grantee institutions, and include the following research mechanisms:

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. The special focus for FY2010 was Breast Cancer Prevention. Funding: Up to \$5M for 3 to 5 years.

[The ligan SDG as a prevention strategy for pre-menopausal women at high risk for development of breast cancer](#)

Mechanism: Promise Grants \$4,500,047
Principal Investigator(s): Carol Fabian, MD
University of Kansas Medical Center Kansas City, KS
Stephen Hursting, MD
University of Texas at Austin Austin, TX
Scientific Area: Chemoprevention

[Preclinical and brief exposure early clinical evaluation of an oral PARP inhibitor for breast cancer prevention in BRCA mutation carriers](#)

Mechanism: Promise Grants \$4,999,953
Principal Investigator(s): Judy Garber, MD, PhD and Kornelia Polyak, MD, PhD
Dana-Farber Cancer Institute Boston, MA
Scientific Area: Chemoprevention

[Chromatin remodeling as a preventive strategy in breast cancer](#)

Mechanism: Promise Grants \$500,000
Principal Investigator(s): Jose Russo, MD
Fox Chase Cancer Center Philadelphia, PA
Scientific Area: Chemoprevention

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 and cell killing of breast cancers by using the VA-deleted oncolytic adenovirus regulated by tumor suppressor microRNA](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): David Curiel, MD, PhD and Hideyo Ugai, MD, PhD
University of Alabama at Birmingham Birmingham, AL
Scientific Area: Targeted Therapies

Pending Execution of Grant Agreements

[Mechanisms for higher recurrence risk in breast cancer survivors](#)

Mechanism: Investigator Initiated Research \$599,935
Principal Investigator(s): John P. Pierce, PhD
University of California at San Diego La Jolla, CA
Scientific Area: Epidemiology and Risk Assessment

[Immunotargeting breast tumors](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Jan Schnitzer, MD
Sidney Kimmel Cancer Center San Diego, CA
Scientific Area: Locoregional Therapies

[Therapy of receptor triple-negative breast cancers by targeting the MYC oncogene](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Andrei Goga, MD, PhD
University of California at San Francisco San Francisco, CA
Scientific Area: Targeted Therapies

[Aberrant glucose metabolism: pathogenic factor and therapeutic target in triple negative breast cancers](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Ann Thor, MD
University of Colorado Denver Aurora, CO
Scientific Area: Endocrine Therapies

[Numb signaling and breast cancer](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Weimin Zhong, PhD
Yale University New Haven, CT
Scientific Area: Diagnostic and Prognostic Biomarkers

[Chromosome arm-specific telomere lengths and breast cancer risk](#)

Mechanism: Investigator Initiated Research \$599,985
Principal Investigator(s): Yun-Ling Zheng, PhD
Georgetown University, Lombardi Comprehensive Cancer Center Washington, DC
Scientific Area: Epidemiology and Risk Assessment

[Potentiating tumor immunity in breast cancer patients using aptamer-targeted Foxp3 siRNA to inactivate regulatory T cells](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Eli Gilboa, PhD
University of Miami School of Medicine Miami, FL
Scientific Area: Immunotherapies

Pending Execution of Grant Agreements

[Analysis of oncostatin M in breast cancer metastasis to bone for the purpose of inhibiting disease progression](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Cheryl Jorcyk, PhD
Boise State University Boise, ID
Scientific Area: Pathobiology

[Role of a novel RAR-beta-5 isoform in the resistance of breast cancer cells to retinoids](#)

Mechanism: Investigator Initiated Research \$577,512
Principal Investigator(s): Konstantin Christov, MD, PhD
University of Illinois at Chicago Chicago, IL
Scientific Area: Chemoprevention

[Topical transdermal therapy for breast cancer prevention using dendrimer nanoparticles for drug delivery](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Seema Khan, MD
Northwestern University Chicago, IL
Seungpyo Hong, MD
University of Illinois at Chicago Chicago, IL
Scientific Area: Localized Chemotherapy

[Regulation of estrogen-independent growth by microRNAs in ER positive breast cancer cells](#)

Mechanism: Investigator Initiated Research \$599,264
Principal Investigator(s): Yin-Yuan Mo, PhD
Southern Illinois University School of Medicine Springfield, IL
Scientific Area: Endocrine Therapies

[Targeting telomerase for the treatment of refractory breast cancers](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Brittney-Shea Herbert, PhD
Indiana University Indianapolis, IN
Scientific Area: Targeted Therapies

[Proposed primary antiangiogenic therapy - computational modeling support](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Rick Rogers, PhD and Michael Retsky, PhD
Harvard University Boston, MA
Scientific Area: Targeted Therapies

[Pre-clinical analysis of a novel combinatorial therapeutic strategy for invasive breast cancer and prevention of recurrence](#)

Mechanism: Investigator Initiated Research \$593,631
Principal Investigator(s): Amy Yee, PhD and Kurtz Paulson, PhD
Tufts University Boston, MA
Scientific Area: Targeted Therapies

Pending Execution of Grant Agreements

[Impact of the telomerase inhibitor PinX1 on the development and/or clinical outcome of breast cancer](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Xiao Zhen Zhou, MD and Kun Ping Lu, MD
Beth Israel Deaconess Medical Center, Boston Boston, MA
Scientific Area: Pathobiology

[Targeting microtentacles on circulating breast tumor cells to reduce metastasis](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Stuart Martin, PhD
University of Maryland at Baltimore Baltimore, MD
Scientific Area: Targeted Therapies

[Novel two-component delivery system based on Her-2/neu receptor internalization strategy](#)

Mechanism: Investigator Initiated Research \$599,492
Principal Investigator(s): Dmitri Artemov, PhD
Johns Hopkins University, School of Medicine Baltimore, MD
Scientific Area: Localized Chemotherapy

[Development of a predictive model for improved, cost-effective breast cancer detection based on biomechanical properties of tissue](#)

Mechanism: Investigator Initiated Research \$599,950
Principal Investigator(s): Neb Duric, PhD
Karmanos Cancer Institute Detroit, MI
Scientific Area: Diagnostic and Prognostic Biomarkers

[Chfr and drug sensitivity of human breast cancer](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Scott Kaufmann, MD, PhD
Mayo Clinic and Foundation Rochester, MN
Scientific Area: Diagnostic and Prognostic Biomarkers

[The role of the immune response in the clinical efficacy of combination trastuzumab and chemotherapy](#)

Mechanism: Investigator Initiated Research \$587,315
Principal Investigator(s): Keith Knutson, PhD
Mayo Clinic and Foundation Rochester, MN
Edith Perez, PhD
Mayo Clinic and Foundation Jacksonville, FL
Scientific Area: Immunotherapies

[Combined modality therapies for the treatment of metastatic breast cancer](#)

Mechanism: Investigator Initiated Research \$599,119
Principal Investigator(s): Jonathan Serody, MD
University of North Carolina at Chapel Hill Chapel Hill, NC
Scientific Area: Immunotherapies

Pending Execution of Grant Agreements

[Site-specific proteasome inhibitors for the treatment of triple-negative breast cancers](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Alexei Kisselev, PhD
Dartmouth Medical School Lebanon, NH
Scientific Area: Targeted Therapies

[Human mammary tumor virus \(HMTV\) and breast cancer](#)

Mechanism: Investigator Initiated Research \$399,912
Principal Investigator(s): James Holland, MD and Beatriz Pogo, MD
Mount Sinai School of Medicine New York, NY
Scientific Area: Epidemiology and Risk Assessment

[Targeting CREB-CBP interaction as anti-breast cancer therapy](#)

Mechanism: Investigator Initiated Research \$599,996
Principal Investigator(s): Xiangshu Xiao, PhD
Oregon Health and Sciences University Portland, OR
Scientific Area: Targeted Therapies

[Mining the breast cancer "stromal proteome":using targeted proteomics to identify novel stromal breast cancer biomarkers](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Michael Lisanti, MD, PhD
Thomas Jefferson University Philadelphia, PA
Scientific Area: Diagnostic and Prognostic Biomarkers

[Co-targeting parathyroid hormone-related protein signaling and osteoclast metabolism to counter breast cancer metastasis to bone](#)

Mechanism: Investigator Initiated Research \$599,904
Principal Investigator(s): Richard Kremer, MD, PhD and Andrew Karaplis, MD, PhD
McGill University, Royal Victoria Hospital Montreal, Quebec, Canada
Scientific Area: Targeted Therapies

[Targeting metabolic processes in breast cancer cells](#)

Mechanism: Investigator Initiated Research \$590,497
Principal Investigator(s): Wilson Miskimins, PhD
Sanford Research/USD Sioux Falls, SD
Scientific Area: Targeted Therapies

[Development of ODAM as a biomarker in breast cancer](#)

Mechanism: Investigator Initiated Research \$575,983
Principal Investigator(s): Daniel Kestler, PhD
University of Tennessee Health Science Center Knoxville, TN
Scientific Area: Diagnostic and Prognostic Biomarkers

Pending Execution of Grant Agreements

[CDK2 is a novel target for triple negative breast cancer](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Khandan Keyomarsi, PhD and Kelly Hunt, PhD
M.D. Anderson Cancer Center, University of Texas Houston, TX
Scientific Area: Targeted Therapies

[LMW-E, a novel indicator of Letrozole resistance in breast cancer](#)

Mechanism: Investigator Initiated Research \$600,000
Principal Investigator(s): Khandan Keyomarsi, PhD and Kelly Hunt, PhD
M.D. Anderson Cancer Center, University of Texas Houston, TX
Scientific Area: Endocrine Therapies

[Role of dicer and BCRP in hormone resistance](#)

Mechanism: Investigator Initiated Research \$599,994
Principal Investigator(s): Suzanne Fuqua, PhD
Baylor College of Medicine Houston, TX
Scientific Area: Endocrine Therapies

[Pharmacodynamic and tissue measures of early breast cancer endocrine sensitivity](#)

Mechanism: Investigator Initiated Research \$599,954
Principal Investigator(s): Hannah Linden, MD and David Mankoff, MD
University of Washington School of Medicine Seattle, WA
Scientific Area: Endocrine Therapies

[Pantoprazole as a modifier of drug resistance: effects on intra-tumoral distribution and efficacy of doxorubicin for treatment of metastatic breast cancer](#)

Mechanism: Investigator Initiated Research \$597,150
Principal Investigator(s): Ian Tannock, MD, PhD
University Health Network, Ontario Cancer Institute Toronto, Ontario, Canada
Scientific Area: Localized Chemotherapy

[Pregnancy-associated breast cancer; timing and mode of diagnosis, tumour characteristics, prognosis and risk factors](#)

Mechanism: Investigator Initiated Research \$568,329
Principal Investigator(s): Mats Lambe, MD, PhD
Karolinska Institute Stockholm, Sweden
Chung-Cheng Hsieh, MD, PhD
University of Massachusetts Medical School Worcester, MA
Scientific Area: Epidemiology and Risk Assessment

Career Catalyst Research—This 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.

Pending Execution of Grant Agreements

[Combination of histone deacetylase inhibitor entinostat and aromatase inhibitor letrozole in ER-negative breast cancer metastasis model](#)

Mechanism: Career Catalyst Research \$449,999
Principal Investigator(s): Gauri Sabnis, PhD
University of Maryland School of Medicine Baltimore, MD
Scientific Area: Endocrine Therapies

[Matriptase-mediated signaling in breast cancer as a target for therapeutic intervention](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): Karin List, PhD
Wayne State University Detroit, MI
Scientific Area: Pathobiology

[The role of ER-beta and endoxifen in the treatment and progression of breast cancer](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): John Hawse, PhD
Mayo Clinic and Foundation, Rochester Rochester, MN
Scientific Area: Endocrine Therapies

[Type igamma phosphatidylinositol phosphate kinase: a Key molecule promoting breast cancer metastasis](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): Kun Ling, PhD
Mayo Clinic and Foundation, Rochester Rochester, MN
Scientific Area: Pathobiology

[Validating CYP2D6 genotype-guided tamoxifen therapy for a multiracial U.S. population](#)

Mechanism: Career Catalyst Research \$449,714
Principal Investigator(s): William Irvin, MD and Lisa Carey, MD
University of North Carolina at Chapel Hill Chapel Hill, NC
Scientific Area: Diagnostic and Prognostic Biomarkers

[Amphiregulin: biomarker evaluation and functional analysis in estrogen receptor positive breast cancer](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): Paraic Kenny, PhD
Albert Einstein College of Medicine at Yeshiva University Bronx, NY
Scientific Area: Diagnostic and Prognostic Biomarkers

[Regulation of the response to cytotoxic chemotherapy by the breast cancer tumor microenvironment](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): Mikala Egeblad, PhD
Cold Spring Harbor Laboratory Cold Spring Harbor, NY
Scientific Area: Pathobiology

Pending Execution of Grant Agreements

[Kinase gene mutations and amplification in breast precursor lesions and progression: potential role in diagnosis and targeted therapy](#)

Mechanism: Career Catalyst Research \$449,850
Principal Investigator(s): Megan Troxell, MD, PhD
Oregon Health and Sciences University Portland, OR
Scientific Area: Diagnostic and Prognostic Biomarkers

[Overcoming resistance to HER2-targeted therapies through inhibition of HER3-induced PI3K activity](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): Rebecca Muraoka-Cook, PhD
Vanderbilt University, School of Medicine Nashville, TN
Scientific Area: Targeted Therapies

[The role of NEDD9 protein in proliferation and metastasis of breast cancer](#)

Mechanism: Career Catalyst Research \$450,000
Principal Investigator(s): Elena Pugacheva, PhD
West Virginia University Morgantown, WV
Scientific Area: Pathobiology

Career Catalyst in Disparities Research—The Career Catalyst in Disparities Research grants seek to foster independent careers in research exploring the basis for differences in breast cancer outcomes and the translation of this research into clinical and public health practice interventions, particularly among junior scientists from populations affected by breast cancer disparities. Funding: \$300,000 for 2 years and a performance-based option for a \$150,000 3rd year.

[Quality, disparities, and breast cancer care in medicaid](#)

Mechanism: Career Catalyst in Disparities Research \$437,998
Principal Investigator(s): Michael Hassett, MD
Dana-Farber Cancer Institute Boston, MA
Scientific Area: Epidemiology and Risk Assessment

[Improving outcomes for older women with breast cancer](#)

Mechanism: Career Catalyst in Disparities Research \$449,984
Principal Investigator(s): Cynthia Owusu Owusu, MD
Case Western Reserve University, School of Medicine Cleveland, OH
Scientific Area: Epidemiology and Risk Assessment

Postdoctoral Fellowship—The Postdoctoral Fellowship grants seek to (a) attract scientists into careers addressing important research questions about breast cancer, (b) expand the skills and expertise of breast cancer researchers in training, and (c) position trainees for independent careers conducting breast cancer research that will directly affect breast cancer patients. Funding: \$60,000 per year for 2 years and a performance-based option for a \$60,000 3rd year.

Pending Execution of Grant Agreements

[Understanding the development of breast cancer: The role of SnoN in the mammary cells function and breast cancer progression](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Kunxin Luo, PhD
University of California at Berkeley Berkeley, CA
Fellow: Juliet Rashidian, PhD
Scientific Area: Oncogenes and Tumor Suppressor Genes

[A combined MRI-dynamic contrast enhanced fluorescence tomography system for breast cancer imaging](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Gultekin Gulsen, PhD
University of California at Irvine Irvine, CA
Fellow: Yuting Lin, PhD
Scientific Area: Detection and Diagnosis

[A strategy for the identification of tumor translocations in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Michael Rosenfeld, PhD
University of California at San Diego La Jolla, CA
Fellow: Maria Cardamone, PhD
Scientific Area: Endocrine Biology and Therapies

[Multiplexed DNA methylation biomarkers as a diagnostic tool for the early detection of breast cancer](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Peter Laird, PhD
University of Southern California Los Angeles, CA
Fellow: Simeen Malik, PhD
Scientific Area: Detection and Diagnosis

[Functional analysis of the DLK1-GTL2 imprinted region in breast stem cell differentiation and tumorigenesis](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Michael Clarke, MD
Stanford University Palo Alto, CA
Fellow: Maider Zabala Ugalde, MD
Scientific Area: Tumor Progression

[Using a novel epigenetic mark to provide fundamental insights into the biology of breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Jessica Tyler, PhD
University of Colorado Health Sciences Center Aurora, CO
Fellow: Ryosuke Ohsawa, PhD
Scientific Area: Diagnostic and Therapeutic Targets

Pending Execution of Grant Agreements

[Early detection of lymphedema using ultrasound elastography](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Tian Liu, PhD
Emory University, Winship Cancer Institute Atlanta, GA
Fellow: Hao Chen, PhD
Scientific Area: Detection and Diagnosis

[Proteomic dissection of DNA damage response in breast cancer cells](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$120,000
Mentor(s): Stephen Kron, PhD
University of Chicago Chicago, IL
Fellow: Satoe Takahashi, PhD
Scientific Area: Genetics and DNA Damage

[Cyclophilin B as a novel target for synthetic antigen binders in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Charles Clevenger, MD, PhD
Northwestern University, Feinberg School of Medicine Chicago, IL
Fellow: Xianke Zeng, MD, PhD
Scientific Area: Immunology and Immunotherapies

[Validating the embryonic morphogen Nodal as a new therapeutic target for breast carcinoma](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$120,000
Mentor(s): Mary Hendrix, PhD
Children's Memorial Hospital Chicago, IL
Fellow: Gina Kirsammer, PhD
Scientific Area: Molecular Biology

[Development of inhibitors targeting p130Cas/BCAR1 family proteins](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Kathrin Kirsch, PhD
Boston University School of Medicine Boston, MA
Fellow: Kumbirnk Joerg, PhD
Scientific Area: Experimental Therapeutics

[Identification of a new STAT3 inhibitor for breast cancer therapy: Mechanistic and preclinical evaluation](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$120,000
Mentor(s): David Frank, PhD
Dana-Farber Cancer Institute Boston, MA
Fellow: Sarah Walker, PhD
Scientific Area: Experimental Therapeutics

Pending Execution of Grant Agreements

[Inhibition of Tumor Endothelial Marker 8 \(TEM-8\) as an anti-angiogenic therapeutic approach for breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Robert Damato, PhD
Children's Hospital, Boston Boston, MA
Fellow: Lorna Cryan, PhD
Scientific Area: Experimental Therapeutics

[Molecular pathways in mesothelial cell differentiation: role in breast tumor progression](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Kornelia Polyak, MD, PhD
Dana-Farber Cancer Institute Boston, MA
Fellow: Ying Su, MD, PhD
Scientific Area: Angiogenesis, Invasion, and Metastasis

[Targeting centrosome clustering as a therapeutic strategy in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): David Pellman, PhD
Dana-Farber Cancer Institute Boston, MA
Fellow: Shang-Yi Chiu, PhD
Scientific Area: Therapeutics

[Fanconi anemia/BRCA pathway and PARP1 inhibition in BRCA1-proficient breast cancers](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Alan D'Andrea, PhD
Dana-Farber Cancer Institute Boston, MA
Fellow: Eunmi Park, PhD
Scientific Area: Therapeutics

[In silico discovery and optimization of a novel estrogen receptor alpha antagonist degradation platform for the treatment of drug resistant breast cancer](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Alan Rigby, PhD
Beth Israel Deaconess Medical Center Boston, MA
Fellow: Kumaran Shanmugasundaram, PhD
Scientific Area: Therapeutics

[The role of HOXB13 in the development of tamoxifen resistance](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Zaver Bhujwalla, PhD
Johns Hopkins University, School of Medicine Baltimore, MD
Fellow: Kideok Jin, PhD
Scientific Area: Experimental Therapeutics

Pending Execution of Grant Agreements

[Phase II study of 5-azacytidine and entinostat \(MS-275\) in patients with advanced breast cancer](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$120,000
Mentor(s): Vered Stearns, MD
Johns Hopkins University, Kimmel Cancer Center Baltimore, MD
Fellow: Roisin Connolly, MD
Scientific Area: Therapeutics

[Prostaglandin E EP1 receptor in breast cancer metastasis and disparities](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Amy Fulton, PhD
University of Maryland at School of Medicine Baltimore, MD
Fellow: Jocelyn Reader, PhD
Scientific Area: Therapeutics

[Evaluation of aldehyde dehydrogenase inhibitors for the prevention and treatment of triple negative breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Angelika Burger, PhD and Fred Miller, PhD
Wayne State University Detroit, MI
Fellow: Fathima Kona, PhD
Scientific Area: Experimental Therapeutics

[Breast cancer therapy by tumor-specific inhibition of DNA-repair proteins](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Shana Sturla, PhD
University of Minnesota at Twin Cities Minneapolis, MN
Fellow: Marina Tanasova, PhD
Scientific Area: Genetics and DNA Damage

[AIB1 \(amplified in breast cancer 1\) is a key regulator of the IGF pathway in triple negative breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Douglas Yee, PhD
University of Minnesota at Twin Cities Minneapolis, MN
Fellow: Annabell Oh, PhD
Scientific Area: Diagnostic and Therapeutic Targets

[Combination therapy for breast cancer using anti-PD-1 antibody and "infrastructure" vaccine](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Keith Knutson, PhD and Peter Wettstein, PhD
Mayo Clinic and Foundation, Rochester Rochester, MN
Fellow: Lavakumar Karyampudi, PhD
Scientific Area: Immunology and Immunotherapies

Pending Execution of Grant Agreements

[Characterization of BRCA2 variants of uncertain significance \(VUS\) using genetic and functional approaches](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Fergus Couch, PhD
Mayo Clinic and Foundation, Rochester Rochester, MN
Fellow: Lucia Guidugli, PhD
Scientific Area: Genetic Risk and Epidemiology

[The role of taspase1 in HER2/Neu driven tumorigenesis](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): James Hsieh, MD, PhD and Matthew Ellis, MD, PhD
Washington University at St. Louis, School of Medicine St Louis, MO
Fellow: Brian Van Tine, MD, PhD
Scientific Area: Growth Factors and Signal Transduction

[Identifying modifiable determinants of cardiovascular disease outcomes among breast cancer survivors](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Marilie Gammon, PhD
University of North Carolina at Chapel Hill Chapel Hill, NC
Fellow: Patrick Bradshaw, PhD
Scientific Area: Genetic Risk and Epidemiology

[Delivery of immune-modulating agents directly to the tumor site in combination with a MUC1 vaccine for the treatment of breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Pinku Mukherjee, PhD
University of North Carolina at Charlotte Charlotte, NC
Fellow: Jennifer Curry, PhD
Scientific Area: Immunology and Immunotherapies

[TGF-beta regulates miR-126/126* expression to promote breast cancer metastasis](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$120,000
Mentor(s): Xiao-Fan Wang, PhD
Duke University Durham, NC
Fellow: Xin Xu, PhD
Scientific Area: Growth Factors and Signal Transduction

[Interactive effects of cruciferous vegetable intake and NAD\(P\)H:quinone oxidoreductase 1 \(NQO1\) functional polymorphisms on breast cancer prognosis](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Christine Ambrosone, PhD
Roswell Park Cancer Institute Buffalo, NY
Fellow: Li Tang, PhD
Scientific Area: Prevention and Risk Reduction

Pending Execution of Grant Agreements

[Identifying oncogenic targets in trastuzumab-refractory HER2-amplified and triple-negative breast cancer: A phase I/II trial of EGFR/HER2 and mTOR inhibition](#)

Mechanism: Postdoctoral Fellowship - Clinical Research \$120,000
Mentor(s): Clifford Hudis, MD
Memorial Sloan-Kettering Cancer Center New York, NY
Fellow: Devika Gajria, MD
Scientific Area: Targeted Therapies

[Characterizing the impact of cancer therapy on fertility and sexual health in women with breast cancer](#)

Mechanism: Postdoctoral Fellowship - Clinical Research \$180,000
Mentor(s): Maura Dickler, MD
Memorial Sloan-Kettering Cancer Center New York, NY
Fellow: Shari Goldfarb, MD
Scientific Area: Epidemiology and Risk Assessment

[Androgen receptor regulates PTEN in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Charis Eng, MD, PhD
Cleveland Clinic Foundation Cleveland, OH
Fellow: Yu Wang, MD, PhD
Scientific Area: Oncogenes and Tumor Suppressor Genes

[A systems biology approach for dissecting microenvironment networks in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Michael Ostrowski, MD, PhD
Ohio State University, College of Medicine Columbus, OH
Fellow: Anand Merchant, MD, PhD
Scientific Area: Angiogenesis, Invasion, and Metastasis

[Non-invasive tissue response monitoring during neoadjuvant chemotherapy using Diffuse Optical Tomography and MRI](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Arjun Yodh, PhD
University of Pennsylvania Philadelphia, PA
Fellow: So Hyun Chung, PhD
Scientific Area: Therapeutics

[Identifying chemosensitivity nodes and small molecule inhibitors regulating breast cancer stem cell proliferation and survival](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$120,000
Mentor(s): John Lazo, PhD
University of Pittsburgh Pittsburgh, PA
Edward Prochownik, PhD
Children's Hospital, Pittsburgh Pittsburgh, PA
Fellow: Fang Zhang, PhD
Scientific Area: Experimental Therapeutics

Pending Execution of Grant Agreements

[Genotype-specific microRNA function in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Michael White, PhD
University of Texas Southwestern Medical Center Dallas, TX
Fellow: Malia Potts, PhD
Scientific Area: Oncogenes and Tumor Suppressor Genes

[Development of annexin A2-targeted curcumin-loaded multifunctional PLGA nanoparticles for breast cancer therapy](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Jamboor Vishwanatha, PhD
University of North Texas Health Science Center Fort Worth, TX
Fellow: Anindita Mukerjee, PhD
Scientific Area: Detection and Diagnosis

[The role of p27kip1 deregulation during cell migration and metastasis](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Catherine Denicourt, PhD
University of Texas Health Science Center at Houston Houston, TX
Fellow: Erica Cassimere, PhD
Scientific Area: Molecular Biology

[Targeting acquired Lapatinib-resistance in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Prahlad Ram, PhD
M.D. Anderson Cancer Center, University of Texas Houston, TX
Fellow: Kakajan Komurov, PhD
Scientific Area: Experimental Therapeutics

[Role of Rab25 in progression of breast cancer](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$180,000
Mentor(s): Gordon Mills, PhD
M.D. Anderson Cancer Center, University of Texas Houston, TX
Fellow: Shreya Mitra, PhD
Scientific Area: Therapeutics

[Nested nanoparticles for chemotherapeutic synergy enhancement in breast cancer](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$178,801
Mentor(s): Mauro Ferrari, PhD
University of Texas Health Science Center at Houston Houston, TX
Fellow: Elvin Blanco, PhD
Scientific Area: Therapeutics

Pending Execution of Grant Agreements

[Targeting microRNAs to alter radiation resistance of breast cancer stem cells](#)

Mechanism: Post Doctoral Fellowship - Translational Research \$179,303
Mentor(s): Wendy Woodward, MD, PhD
M.D. Anderson Cancer Center, University of Texas Houston, TX
Fellow: Bisrat Debeb, MD, PhD
Scientific Area: Therapeutics

[Targeting LC3-SQSTM1/p62 signaling axis to treat apoptosis-resistant and metastatic breast cancers](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Pothana Saikumar, PhD and Rajeshwar Tekmal, PhD
University of Texas Health Science Center at San Antonio San Antonio, TX
Fellow: Prajjal Singha, PhD
Scientific Area: Experimental Therapeutics

[The role of c-Src in ErbB2-driven mammary tumorigenesis and metastasis](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): William Muller, PhD
McGill University Montreal, Quebec, Canada
Fellow: Harvey Smith, PhD
Scientific Area: Oncogenes and Tumor Suppressor Genes

[Investigation into the role of met, a receptor tyrosine kinase, in the development of basal-like breast cancer](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$180,000
Mentor(s): Morag Park, PhD
McGill University Montreal, Quebec, Canada
Fellow: Jennifer Knight, PhD
Scientific Area: Oncogenes and Tumor Suppressor Genes

[The role of Ret receptor in breast cancer: implications of Ret activation in endocrine resistance](#)

Mechanism: Post Doctoral Fellowship - Basic Research \$179,000
Mentor(s): Nancy Hynes, PhD
Friedrich Miescher Institute for Biomedical Research Basel, Switzerland
Fellow: Albana Gattelli, PhD
Scientific Area: Growth Factors and Signal Transduction

Pending Execution of Grant Agreements

Post-Baccalaureate Training in Disparities Research—These grants seek to (a) attract individuals from populations affected by disparities in breast cancer outcomes into careers seeking to understand and eliminate these disparities; (b) provide the tools and environment in which students very early in their career can begin to define meaningful career paths focused on addressing disparities in breast cancer outcomes; and (c) empower these students with the analytic, research, scientific, clinical, and public health skills critical to effectively (1) explore the basis for differences in breast cancer outcomes; and (2) translate research discoveries into clinical and public health practice to eliminate disparities in breast cancer outcomes. This grant is intended to establish a training program headed by a mentor(s) to support qualified individuals who are dedicated to pursuing research in breast cancer disparities. Funding: \$135,000 per student, per year, over 3 years.

[AYUDA: a training program to improve breast cancer outcomes among Latina patients](#)

Mechanism: Post-Baccalaureate Training in Disparities Research \$270,000
Principal Investigator(s): Evelinn Borrayo, PhD
University of Colorado at Denver Denver, CO
Peter Raich, PhD
Denver Health Denver, CO
Scientific Area: Nutritional, Behavioral and Lifestyle Prevention

[Interdisciplinary research training in breast cancer disparities](#)

Mechanism: Post-Baccalaureate Training in Disparities Research \$180,000
Principal Investigator(s): M. Tish Knobf, PhD and Lyndsay Harris, PhD
Yale University New Haven, CT
Scientific Area: Epidemiology and Risk Assessment

[American Indian Health Research & Education Alliance Master in Public Health Program in American Indian breast cancer disparities](#)

Mechanism: Post-Baccalaureate Training in Disparities Research \$405,000
Principal Investigator(s): Christine Daley, PhD
University of Kansas Medical Center Kansas City, KS
Scientific Area: Nutritional, Behavioral and Lifestyle Prevention

[Boston University Women's Health Unit Training Program: patient navigation and clinical intervention research to eliminate breast cancer health disparities](#)

Mechanism: Post-Baccalaureate Training in Disparities Research \$405,000
Principal Investigator(s): Karen Freund, MD
Boston University School of Medicine Boston, MA
Scientific Area: Nutritional, Behavioral and Lifestyle Prevention

[Breast cancer disparities in rural Appalachia](#)

Mechanism: Post-Baccalaureate Training in Disparities Research \$269,936
Principal Investigator(s): Roger Anderson, PhD
Pennsylvania State University College of Medicine Hershey, PA
Marianne Hillemeier, PhD
Pennsylvania State University University Park, PA
Scientific Area: Epidemiology and Risk Assessment

Public Abstracts

PI Name: Carol Fabian, MD

Institution: University of Kansas Medical Center

Mechanism: Promise Grants

Application Title: The lignan SDG as a prevention strategy for pre-menopausal women at high risk for development of breast cancer

Abstract

Plant lignans are chemical compounds found in edible fiber rich plants, which may act as anti-estrogens when converted by bacteria in the colon into their mammalian counterparts. Secoisolariciresinol (SECO) is one such lignan found in high concentrations in flaxseed. It is converted to enterolactone (ENL) and enterodiol (EDL) of which the former is probably the most bioactive component. Studies in rats and mice and case control studies in humans suggest that either SECO or flaxseed may reduce risk for breast cancer, particularly in younger women who are still premenopausal and producing appreciable levels of estrogen. However, a definitive trial assessing cancer incidence after women have been randomized to SECO or flax vs a placebo has not yet been performed. The practical advantage of flaxseed or its lignan component, SECO, are safety, minimal side effects, and low cost. SECO when given as the diglycoside (SDG) seems to cause fewer problems with gas and indigestion than raw flaxseed.

Our group has conducted a 12 month study in which daily consumption of SECO diglycoside (SDG) was associated with a ten-fold elevation in lignan blood levels (reflecting excellent compliance with SDG) and minimal side effects. We observed a decrease in indicators of breast cell growth (Ki-67) in 80% of participants and a reduction in the proportion of women with precancerous (atypical) cells in breast tissue obtained by a well-accepted minimally invasive sampling procedure, random peri-areolar fine needle aspiration (RPFNA). Increase in cell growth particularly in areas of pre-cancerous change, are associated with increased risk for development of breast cancer. Thus, when we see a reduction in these properties, it is suggestive of reduced risk, although that has yet to be proven.

Our Hypothesis: SDG will reduce Ki-67 and decrease atypia in benign breast tissue from premenopausal women, as well as breast tumor incidence and number in animal studies.

Methods: We plan to conduct a multi-institutional, trial of placebo vs SDG in premenopausal women ages 25-49 at moderate to high risk for development of breast cancer to confirm the apparent reduction in cell growth and precancerous cells observed in our pilot study. Since reduction in cancer incidence is beyond the scope of our proposed trial, we plan corresponding studies in animals that develop estrogen dependent (ER+) or independent (ER-) breast cancers to determine if achieving similar blood levels of lignans as we achieved in our pilot trial will reduce cell growth and precancerous cells and ultimately incidence of cancer. SDG's affect on additional markers of risk including hormones, growth factors, inflammatory factors and mammographic density will be assessed along with measures of quality of life and relief from breast discomfort, and maintenance of fertility, particularly in young women.

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Expected Outcomes: 12 months of SDG compared to placebo is expected to decrease Ki-67 and the proportion of women with precancerous cells in their sampled breast tissue without an adverse effect on quality of life or fertility. SDG is also expected to decrease cell growth and precancerous cells at an early follow-up time point in animal studies, translating to reduced tumor incidence at a later follow-up time point. Since SDG is widely available as a food supplement, favorable effects on human risk biomarkers combined with reduction in cancer incidence in animal tumor models could result in decreased cancer incidence within the decade as Phase III cancer incidence trials would not necessarily need to be performed. Exploratory biomarker studies to delineate SDG's mechanism of action are suggested should the animal or human studies appear favorable.

Future Implications: This option for a well tolerated, immediate available breast cancer prevention agent targeting both major types of breast cancer has the potential to change the landscape of breast cancer prevention now and in the future.

Pending Execution of Grant Agreements

PI Name: Judy Garber, MD, PhD

Institution: Dana-Farber Cancer Institute

Mechanism: Promise Grants

Application Title: Preclinical and brief exposure early clinical evaluation of an oral PARP inhibitor for breast cancer prevention in BRCA mutation carriers

Abstract

For the past 15 years, we have been able to identify women at remarkably high breast cancer risk because they carry a mutation in one copy of their BRCA1 or BRCA2 genes. Because many brave women and their families participated in research in those early days, we can now provide estimates of the risk that a woman with a BRCA mutation will develop breast and/or ovarian cancer, but we cannot tell a woman when cancer might develop. Women with BRCA mutations can now try to manage their increased cancer risks with more intense monitoring (adding breast MRI to mammograms and breast exams), and reducing hormonal stimulation (removing ovaries and fallopian tubes reduces ovarian cancer risk by 90% or more, and reduces breast cancer risk if performed well before menopause). Prophylactic bilateral mastectomy reduces breast cancer risk by 90% or more. However, despite improvements in reconstructive techniques, surgery is a difficult and often unacceptable for many women. Most mutations have been passed down for generations, and will burden the daughters and granddaughters of women whose mutations are identified today.

There is new excitement about the novel agents called PARP inhibitors. Scientists recognized that these agents could be particularly active in killing cancer cells in women with inherited BRCA mutations. This activity was expected because: 1) BRCA1 and BRCA2 are critical for repairing mistakes in the DNA (cell "blueprint") which are common in dividing cells; 2) in the normal tissue of women with BRCA mutations, there is one mutated copy of BRCA1 or 2, but the second copy of the gene is normal, and its presence is enough to maintain normal cell function; 3) in breast (and ovarian) cancers of women with BRCA mutations, there is early loss of the normal copy of the gene, and the tumor cells cannot fix one common type of DNA error, leading to the accumulation of mistakes, and ultimately cancer. Tumor cells must then rely on other DNA repair pathways to keep from collecting so much damage that they must self-destruct; 4) PARP inhibitors block back-up DNA repair pathways, so the cancer cells collect DNA damage and do self-destruct, but normal cells are not affected. This would be a good drug for prevention and treatment - one that kills tumor cells without damaging normal cells.

A PARP inhibitor in an oral form is a possible prevention agent, as women might be willing to take it. Early studies of mice engineered to carry a BRCA mutation in their mammary tissue show that a PARP inhibitor can delay the development of tumors the mice get over time. An agent that is safe, with few side effects, that could reliably reduce breast cancer (and simultaneously ovarian cancer) risk in high risk BRCA women or delay breast cancer until they are much older would be very exciting indeed!

Pending Execution of Grant Agreements

We will approach this carefully and methodically. Our mouse chemoprevention research team will study three PARP inhibitors given orally, and compare them to see which can best reduce the development of tumors in mice engineered to have BRCA1 mutation in their mammary tissue, and to look for potential serious side effects.

We have also designed a group of integrated studies to carefully examine an oral PARP inhibitor that has been given to BRCA mutation carriers in breast and ovarian cancer treatment trials to examine its potential role in breast cancer prevention. Because the PARP inhibitors are still given only in research trials for cancer treatment, we don't have the kind of safety data about them that we usually require for a medication used for prevention. Therefore, we have designed a study in which women with BRCA mutations take the agent twice a day for only 4 weeks – a short time – just before they are having their previously scheduled prophylactic mastectomies. We will ask these women to let us take a blood sample, a sample of breast tissue with a needle and syringe (called RPFNA, a technique for sampling breast cells), and ask them to consider an optional breast biopsy done by the radiologist a month before their planned surgery. We will monitor them carefully for the 4 weeks before surgery when they are taking the medication, then at surgery, we will take their healthy breast tissue for research (after tissue needed for the pathologist for clinical examination is taken), and we'll take more blood, and another RPFNA (under anesthesia). Women participating in the study will begin at the previously studied dose of 200mg; the dose will be reduced for the next group of women until we establish the lowest dose at which its activity can be measured, so that we can use the lowest effective dose going forward. We measure levels of the drug and how well it inhibits PARP in white blood cells. We will give the breast tissue to our scientists to have them look for the effects of the PARP inhibitor on important genes and proteins in the breast tissue and breast stem cells, which we think are affected preferentially by the PARP inhibitor. We will look for cells that have lost the 2nd copy of BRCA1 or 2 without other changes. Dr. Seewaldt will take the biomarkers identified in projects 3 and 4 and adapt them for use with RPFNA, a technique that could be used to monitor women in a larger prevention trial to rapidly tell if the medication were hitting the target in an individual.

We hope that our project will provide information that could lead to a large, definitive cancer prevention trial with a PARP inhibitor for women with BRCA mutations, providing a safe, nonsurgical risk-reducing strategy for women at high risk of breast cancer.

Pending Execution of Grant Agreements

PI Name: Jose Russo, MD

Institution: Fox Chase Cancer Center

Mechanism: Promise Grants

Application Title: Chromatin remodeling as a preventive strategy in breast cancer

Abstract

Background, Rationale and Significance: Breast cancer, which is most frequently diagnosed in postmenopausal Caucasian women, has recently increased in incidence in premenopausal women. Diagnosis of breast cancer before age 40 is prevalent among carriers of BRCA1 or BRCA2 deleterious mutations and in African American women. Both groups of women develop more aggressive tumors that lack estrogen (ER) and progesterone (PR) receptors and Her2, being classified as basal-like triple negative tumors. These tumor characteristics make these patients ineligible for hormone therapy, such as Tamoxifen, aromatase inhibitors or immunotherapy like Trastuzumab. In addition, current guidelines for reducing breast cancer risk that are applicable only for carriers of BRCA1 or BRCA2 deleterious mutations, recommend multi-modality screening and breast and ovary removal (prophylactic mastectomy and oophorectomy) at a young age. Unfortunately, even if performed at a young age, the breast specimens obtained from BRCA1/2 carriers already contain preinvasive lesions or even invasive carcinomas, a clear indication that any preventive measures have to be implemented several years earlier. The fact that women whose first pregnancy was completed before age 24 or had multiple pregnancies and breastfed there is lifetime decrease in breast cancer risk indicates that pregnancy has successfully has permanently modified the structure of the breast. We have found that pregnancy confers protection through the stimulus of hormones produced by the baby and its placenta, mainly human chorionic gonadotropin (hCG). It profoundly modifies the genomic profile of the mammary epithelium in both women and rats, imprinting a permanent “genomic signature” that is characterized by the activation or silencing of transcription factors and chromatin remodeling genes whose products are required for controlling recruitment of protein/protein or DNA/protein interactions. We have found that a small portion of hCG, a peptide that we have called PPFC-897-81-95 acts as the complete hormone and differentiates human breast epithelial cells in the same manner that does hCG. In this application we propose to test the hypothesis that this peptide can be used for preventing breast cancer by changing the genomic signature of breast cells from high to low susceptibility to develop cancer. Because the changes induced by pregnancy and hCG treatment are permanent and confer the same degree of protection, we expect that treatment with this peptide will induce a genomic signature of protection without the need of administering prolonged treatments. The imprinting of a permanent “genomic signature” will indicate that activation or silencing of transcription factors and chromatin remodeling genes whose products are required for controlling recruitment of protein/protein or DNA/protein interactions have occurred. and will serve as a biomarker indicative of an overall lifetime decreased breast cancer risk. **Study Objective/ Hypothesis:** The hypothesis of this application is that PPFC-879-81-95, a novel fifteen amino acid peptide that we have discovered exerts a differentiating effect on the breast epithelium similar to that induced by both pregnancy and r-hCG, is able to induce terminal differentiation of the breast that will ultimately shift its genomic signature from one of high susceptibility to one of refractoriness

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to carcinogenesis. This effect is exerted through activation of specific differentiation-associated genomic and epigenomic pathways triggered by the binding of this peptide to the chorionic gonadotropin receptor, activating mechanisms controlling chromatin remodeling and ultimately expressing a genomic signature of prevention. Our specific aims are: 1. To determine if the peptide PFC-879-81-95 inhibits or reverts the expression of transformation phenotypes induced by 17 beta? estradiol in human breast epithelial cells as a result of activation of differentiation-associated pathways resulting from activation of the cells' genomic profile or induction of epigenetic changes associated with chromatin remodeling. 2. To determine the specificity and efficiency of peptide PFC-879-81-95 in activating chromatin remodeling pathways by comparisons with promissory short peptide sequences such as the newly designed PFC-879-91-110 peptide, for achieving optimal degree of cell differentiation. 3. To identify which one of the peptides studied or identified in specific aims 1 and 2, respectively, induces a degree of mammary gland differentiation equal or more efficient than that induced by a full term pregnancy, or treatment of virgin rats with r-hCG or estrogen/progesterone pellets, resulting in maximal inhibition of chemically induced mammary carcinogenesis. Specific aim 4: i. To establish safety and pharmacokinetics of administration of a fifteen amino acid beta hCG peptide identified in specific aims 1, 2 and 3 in a Phase I study. ii. To establish proof of principle in a randomized phase II trial that the induction of differentiation by treatment with a fifteen amino acid beta hCG peptide will revert a "high risk" to a "low risk" signature, which would serve as a biomarker indicative of decreased breast cancer risk. Project responsiveness to RFA: This application is highly responsive to the RFA by proposing to utilize physiological mechanisms of breast differentiation for preventing cancer by reprogramming breast cell through chromatin remodeling with small peptides, which represent novel and efficacious tools for implementing preventive measures in large populations for eliminating breast cancer before the end of the second decade of the 21st century.

Pending Execution of Grant Agreements

PI Name: David Curiel, MD, PhD

Institution: University of Alabama at Birmingham

Mechanism: Investigator Initiated Research

Application Title: Inhibition and cell killing of breast cancers by using the VA-deleted oncolytic adenovirus regulated by tumor suppressor microRNA

Abstract

Public Abstract

Breast cancer is the second most common cause of cancer deaths in women in the United States (US) and incidence rates have continued to increase since 1980. The rate of developing invasive breast cancer at some time in women in the US is 1 in 8 (about 13%). In 2009, an estimated 192,370 new cases of invasive breast cancer are expected to be diagnosed in women in the US. Approximately 40% of breast cancer patients show resistance to standard chemotherapy, resulting in tumors that will reemerge and subsequently metastasize to lung, pleura, liver and bone. Therefore, breast cancer therapy requires novel medicines to completely eradicate both primary and metastatic breast cancers. This novel medicine should target and eliminate breast cancer cells only.

Genetic engineering has made it possible to tailor adenoviruses for the purposes of cancer therapy. Genetically engineered adenoviruses can distinguish between different types of cells representing a promising attractive drug for breast cancer therapy. We have already developed adenovirus as a medicine capable of distinguishing between breast cancers and normal cells. The next step is to regulate production of genetically engineered adenovirus and cell killing by genetic engineered adenovirus in breast cancer cells for safety. This result will lead to the regression of disease or cure with minimal or no side effects.

Recently, a new class of molecules has been discovered called microRNAs (miRNAs). These molecules properly control the expression of a large number of cellular proteins. This mechanism by which miRNAs regulate the expression of cellular proteins is called "RNA interference (RNAi)". Therefore, recent reports suggest that miRNAs can function not only as novel biomarkers for disease diagnostics but also as a novel factor to control gene expression of cellular proteins. Recent reports of breast cancer suggest that attenuation or loss of miRNA expression is often observed in primary and metastatic breast cancer cells as compared with normal breast cells. Thus, we propose that miRNA biology to properly control protein expression can be exploited to develop adenovirus-based therapy with minimal or no side effects.

Despite the many potential advantages of adenoviral vectors for cancer gene therapy, full utility of the current adenovirus vector would be limited for RNAi applications due to expression of the functional genes that inhibit RNAi mechanism mediated by miRNAs. Therefore, the current adenovirus is not suitable for breast cancer therapy by RNAi application mediated by miRNAs. Thus, it is clear that new types of adenovirus vectors are required for the RNAi application through miRNAs. Of note, an adenoviral vector with a deletion of genes allowing RNAi machinery to function would be an ideal platform for RNAi.

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In this research proposal, we hypothesize that a novel adenoviral vector with a deletion of genes allowing RNAi machinery to function is suitable for RNAi application and modification for miRNA application in the adenoviral vector more effectively kill breast cancer cells with minimal or no side effects. To test our hypotheses, we will delete the adenoviral RNAi inhibitory genes from the adenoviral genome for RNAi application and evaluate the ability of the RNAi application (Specific aim I). While miRNA is detected in normal breast cells, attenuation or loss of miRNA expression is often observed in primary and metastatic breast cancer cells. Thus, miRNA-regulated adenovirus will be not produced by inhibition of miRNA in normal breast cells. In contrast to normal cells, miRNA-regulated adenovirus will be produced in primary and metastatic breast cancer cells with lack of miRNA. In specific aim II, we will assess effectiveness of microRNA-regulated adenovirus for breast cancer therapy in vitro. In addition, we propose to validate activity of cell killing of primary tumor and inhibition of metastatic tumor by the oncolytic adenovirus in vivo (Specific aim III).

The overall goal of this research proposal will provide a novel technology for the RNAi application and therapeutic medicines to eradicate both primary and metastatic breast tumors with minimal or no side effects for breast cancer therapy.

This adenovirus designed based on adenoviral biology maximizes the functionality and mobility of adenovirus for the RNAi application, and the new information of adenoviral biology which is obtained from this research proposal will enhance the treatment of cancer therapy using adenovirus. In addition, the application of miRNAs will uniquely advance the treatment of breast cancer due to properly regulated expression of adenoviral proteins. Information related to miRNAs associated with breast cancer will contribute to the understanding of the generation of breast tumors. A novel therapeutic agent designed for human breast cancers could have significant potential to reduce and cure human breast cancers.

Pending Execution of Grant Agreements

PI Name: John P Pierce, PhD

Institution: University of California at San Diego

Mechanism: Investigator Initiated Research

Application Title: Mechanisms for higher recurrence risk in breast cancer survivors

Abstract

The numbers of women who have survived a diagnosis of breast cancer in the United States is increasing. Women with early stage invasive disease are at significantly increased risk for recurrent disease and untimely death and this risk continues into the second decade following diagnosis. The Women's Healthy Eating and Living (WHEL) study identified a number of sub-groups who were at significantly higher risk than most women for these additional breast cancer events and early death. These included women who had poor physical health scores on a quality of life measure, women who had comorbidities and women who did not have hot flashes shortly after treatment. It is possible that the higher risk in each of these sub-groups was brought about by higher circulating estrogen concentrations. In the WHEL study, even though postmenopausal women have very low levels of circulating estradiol, differences in these concentrations was associated with poorer prognosis. If these small differences are critical to prognosis, targeted lifestyle and pharmaceutical therapy may be able to reduce this added risk.

Study hypothesis:

The study will test whether all of the higher rates of secondary breast cancer events and/or death in postmenopausal breast cancer survivors in the WHEL study were mediated by higher circulating estradiol concentrations

How it advances our understanding of breast cancer and will lead to reductions in incidence and /or mortality:

There have been multiple studies that have identified risk of additional breast cancer events following diagnosis with breast cancer. The WHEL study is unique in that it collected and stored blood samples throughout the study and these allow investigators to go back and study the biology associated with the additional risk. By using these unique multiple samples in such a large study, this proposal will be able to identify how central circulating estradiol concentrations are to the biology of progression in women with early stage invasive breast cancer. Should they be central to the risk of progression,

Importance of this research to patients with cancer:

Sub-groups of patients with early stage breast cancer are at significant risk for recurrent disease and death, however, currently there is no single measure of the level of risk. This study offers the possibility that circulating estradiol concentrations may provide such an indicator of risk. Further, if this is the case, there are known lifestyle and pharmaceutical interventions that can be tested to demonstrate that the risk can be reduced.

Pending Execution of Grant Agreements

PI Name: Jan Schnitzer, MD

Institution: Sidney Kimmel Cancer Center

Mechanism: Investigator Initiated Research

Application Title: Immunotargeting breast tumors

Abstract

Breast cancer is one of the most common cancers in women; the National Cancer Institute reported over 180,000 new cases and 40,000 deaths in the United States during 2008. Most breast cancer is treated with surgical resection followed by chemotherapy, radiotherapy, hormonal therapy, and/or biological therapy. These treatments can lead to a barrage of long-lasting side effects. Additionally, there is currently no effective cure for advanced breast cancer or recurrent breast cancer. Clearly, more effective therapies with fewer side effects are needed. Specifically targeting therapeutics to the diseased tissue may fulfill both goals.

All blood vessels are lined by a thin layer of endothelial cells that act, in part, to control vessel permeability. These cells express different proteins depending on their location in the body. When these proteins extend into the blood, they can act as a “zip code” to target intravenously injected antibodies to the blood vessels of a single tissue. We have discovered that endothelial cells are studded with large numbers of small transport vesicles called caveolae that can rapidly shuttle material out of the blood and pump it into the underlying tissue. Surprisingly, tumors appear to be a distinct type of tissue and express unique “zip code” proteins. One tumor-induced marker is Annexin A1. In a rat model of metastatic breast cancer, Annexin A1 antibodies could specifically target tumors in vivo. When these antibodies were labeled with small radioactive particles, a single injection drastically increased animal survival and eradicated tumors. Annexin A1 is also specifically expressed in vascular endothelial cells from many types of human breast tumors, including metastatic tumors, but lacking from the vasculature in nearby healthy tissue.

It is difficult to deliver clinically useful doses of drugs to tumors. Large drugs are trapped in the blood and cannot get past the endothelium to penetrate into tumors where they can be effective. Small drugs can penetrate throughout the body and are rapidly diluted. Thus, high doses have to be given for any drug to reach the tumor. Targeting caveolae offers an exciting, novel alternative because antibodies are rapidly pumped out of the blood and into the tumors. The antibodies only go to the tumors and are actually concentrated in the tumors. Antibodies can be easily linked to drugs or radioactive particles. This would allow the cargo to specifically accumulate in tumors. This will drastically reduce the chance that they can harm non-tumor cells in the body and increase the safety of administered drugs. Together, these factors suggest that targeting caveolae provides a novel pathway into tumors that can be exploited to pump therapies to the precise location they need to be. This is a significant advance over current therapies that simply cannot penetrate actively and specifically into tumors.

We now need to test this novel strategy to determine how effective targeting tumor caveolae can be. We hypothesize that targeting antibodies to caveolae will allow the antibodies, and

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anything attached, to be rapidly pumped into tumors. This novel pathway will concentrate therapies in the tumors and be much more effective than untargeted therapies that are trapped in the blood and cannot reach the tumors. To test this hypothesis, we determine if radioactive antibodies can effectively destroy breast tumors. We will use several different types of radioactive particles, as well as different doses to optimize this therapy. Targeted radiation is safe because only small amounts are injected and the radiation only collects in the tumor. However, alternative treatments are needed for situations in which radiation is not advised. One alternative we will test is linking anti-cancer drugs to antibodies. We have linked Cisplatin, a commonly used drug, to antibodies and will determine if targeting this drug to tumors makes it more effective and safe than untargeted drugs. Finally, we will further test the safety of antibody therapy by doing toxicology studies on a range of doses.

At every stage from diagnosis through treatment, targeting caveolae can positively impact breast cancer treatment. Early detection is critical to improving survival rates. Because antibodies that target caveolae are concentrated in tumors, it becomes possible to detect the tumors at a much earlier stage. Targeted therapy could also be used to shrink tumors before surgery. By concentrating radioactive molecules or drugs like Cisplatin within tumors, treatments should be more effective and have fewer side effects. Though the incidence rate of breast cancer is approximately equal between Caucasian and African American women, the survival rate is lower for African Americans. This may be due in part to more aggressive forms of breast cancer developing in African American women. More effective therapies, such as radiotherapy directly targeted to the tumor cells, may help to close this disparity by offering another choice for aggressive treatment. Thus, this proposal can have far-reaching effects on how breast cancer is diagnosed and treated, ultimately increasing survival and decreasing the impact on patients' lives. We expect to begin a human clinical trial within three years, and if successful, have a new drug on the market in under 10 years. If the response of human breast cancer patients is anything like what we see in rodent models of breast cancer, we have great hope that this therapy can drastically decrease mortality within the next decade.

Pending Execution of Grant Agreements

PI Name: Andrei Goga, MD, PhD

Institution: University of California at San Francisco

Mechanism: Investigator Initiated Research

Application Title: Therapy of receptor triple-negative breast cancers by targeting the MYC oncogene

Abstract

Human breast cancers that lack expression of the estrogen or progesterone hormone receptors and which do not over-express the growth factor receptor ERBB2 are known as receptor triple-negative cancers. Triple-negative breast cancers are often highly proliferative, poorly differentiated and aggressive tumors and portend a poor prognosis. Owing to their lack of receptor expression, no rational targeted therapeutics against triple-negative breast cancers currently exists, and they thus represent amongst the most difficult to treat types of breast cancer. Our long term objectives are to define the ways by which receptor triple-negative breast cancers survive and grow. We also seek to identify therapeutics that can selectively target this clinically challenging subset of human breast cancers but which do not kill normal cells.

The central hypothesis that will be tested in this proposal is that the MYC cancer gene is present at high levels in triple negative breast cancers, that it is responsible for the growth of these tumor cells, and the MYC is needed for triple-negative tumors to survive.

Important corollaries that will be examined are: 1) If MYC is highly expressed in triple-negative breast cancers, then perhaps we can take advantage of this finding to use small molecules that can selectively kill these cells. In our prior work we discovered that human cells with high-levels of MYC can be preferentially killed by small molecule inhibitors of cell cycle kinases, called CDKs, but these inhibitors do not kill normal cells or those with low levels of MYC (Goga, et. al, Nature Medicine, 2007). We will test if small molecule CDK inhibitors can be used to selectively kill triple-negative breast cancer cells. Our studies will include testing some new and promising CDK inhibitor compounds that are currently in early phase clinical trials. If our studies confirm the activity of these compounds against triple negative breast tumor cells then these compounds, that are already under clinical investigation, could be rapidly tested in patients with triple-negative breast cancers. Thus, the time from confirming our hypothesis to clinical deployment of these compounds for the treatment of patients could be swift.

2) If high-levels of MYC are required for the maintenance of triple-negative tumors, then direct inhibition or "knock-down" of MYC in triple-negative tumor cells may result in blocking the growth or even causing the death of these cells. We will use novel small inhibitory RNA molecules delivered in specially formulated particles that will allow for the depletion of the MYC gene in triple-negative breast cell lines. We will thus determine if MYC is truly required for the growth and survival of this clinically most challenging set of tumors.

Our general strategy is to couple insights gained from the analysis of primary patient samples as part of an ongoing breast cancer clinical trial with high-throughput analysis of ~

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40 human breast cell lines for which gene expression and phenotypic characterization has been previously performed.

This project should uniquely advance our knowledge of triple-negative breast cancers by examining the importance for the MYC gene as a key factor that allows for the growth and survival of this tumor type. If confirmed, the MYC gene could be used both as a new biomarker as well as a target for therapy of triple-negative tumors.

Finally, this project has direct relevance for the treatment of patients with triple-negative breast cancers. Two different novel therapeutics, small molecule CDK inhibitors and selective small RNA inhibitors that target MYC will be tested for their ability to kill triple-negative breast tumors. The same compounds could be used in early phase clinical trials in patients with triple-negative breast tumors.

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PI Name: Ann Thor, MD

Institution: University of Colorado Denver

Mechanism: Investigator Initiated Research

Application Title: Aberrant glucose metabolism: pathogenic factor and therapeutic target in triple negative breast cancers

Abstract

Breast cancers occur at a higher frequency in Caucasian (C) women as compared to ethnic minorities, although the mortality rate is highest amongst African Americans (AA). This survival disparity is particularly profound in pre-menopausal women, where AA have a breast cancer associated death rate 77% higher than Caucasians. Young AA women more frequently develop the most aggressive form of breast cancer, known as 'triple negative'. Epidemiological studies have shown that obesity, type II diabetes and breast feeding practices account for up to 2/3 of TN breast cancers amongst young AA women. These co-morbidities alter host glucose and fructose metabolism, elevating their levels in serum and promoting TN breast cancer development and survival. Unlike other forms of breast cancer, TN cells appear relatively resistant to the pro-growth effects of insulin.

We have shown that the growth and survival of TN cells are overly dependent on the utilization of sugars (glucose, fructose, which are metabolized using a special cancer associated energy pathway known as aerobic glycolysis) and that this defect can be at least partially abrogated by the anti-diabetic agent metformin. It reduces cell growth and induces cell death in TN breast cancer cells. Our data suggests that metformin may be more efficacious against TN cancer cells derived from women of AA heritage, suggesting inheritable (genetic) processes also influence sugar uptake or metabolism to support the growth of TN cancers. In order to develop novel treatment paradigms derived from these studies, we will focus in on the biological and molecular effects of glucose, fructose and metformin, the proteins involved in the uptake and transport of these sugars, aerobic glycolysis and genetic factors.

This grant application is based on three hypotheses 1) TN breast cancer cells are particularly addicted to the sugars glucose and fructose, which provide energy and building block molecules through specialized metabolic processes used more frequently by cancers than normal cells; 2) The widely used, low toxicity anti-diabetic drug metformin reduces TN cell sugar addiction, inhibits cellular replication (tumor growth) and induces TN cell death; and 3) TN cells derived from AA women will show greater sensitivity to metformin. Understanding the mechanism behind these genetic/ethnic differences will allow us to develop treatment protocols that maximize the effect for women of all races with TN disease. Our scientific plan has three major aims 1) studies of glucose, fructose, metformin and EGFR on TN molecular and cellular biology; 2) determination of the role of specific transporter molecules and processes, and their role in sugar uptake and metabolism that promotes TN cancer; 3) Identification and study of specific small RNA molecules that can alter protein production and function, thereby contributing to changes in sugar uptake and metabolism in TN cancer cells.

The committed investigational team includes a Principle Investigator, Ann Thor M.D. who is a pathologist with extensive experience studying dietary factors, hormones and metformin, Steve Anderson Ph.D., who is an expert on breast cancer molecular biology,

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sugar and fat metabolism, glucose transport and utilization and Jennifer Richer Ph.D., who studies breast and endometrial cancers and is an expert in miRNA and their role in human cancers. This proposal is unique, because it selectively focuses on the effects of common sugars, glucose and fructose, on TN breast cancer. High levels of these sugars are found in processed, sweetened and fast-foods. Minorities and lower socioeconomic subgroups consume more of these foods and more frequently develop obesity and diabetes. In young AA women, this triad appears particularly deleterious and promotes TN breast cancer. Because these risk factors may be modified by diet, exercise, cultural shifts and the drug metformin, these studies may set the stage for a better understanding of this terrible form of breast cancer and significantly improve the survival of women who develop the disease, leading to a reduction in TN breast cancer and mortality.

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PI Name: Weimin Zhong, PhD

Institution: Yale University

Mechanism: Investigator Initiated Research

Application Title: Numb signaling and breast cancer

Abstract

A key effort in cancer research is to identify molecular signatures that can guide treatment and predict outcome. A recent study reports an extremely interesting finding that connects the level of a protein called “Numb” to resistance of breast cancers to chemotherapy and prognosis of patients. We have been studying the involvement of Numb, and a related and functionally redundant protein called “Numbl” or “Numbl”, in regulating the behavior of stem cells. The goals of this application are to use the two proteins, as well as an essential partner of Numb and Numbl called “ACBD3”, as entry point to explore the connection between stem cells and breast cancer. Based on recent findings from our laboratory, we propose that breast cancers with lower Numb levels have more cancer stem cells and that eliminating Numb-ACBD3 activity can block breast cancer initiation and progression. We seek to identify predictors of breast cancer prognosis and explore a novel strategy that attempts to treat breast cancer by forcing cancer stem cells to undergo divisions that eliminates themselves.

A fundamental issue in the biology of breast cancer, as well as other cancers, is why killing the vast majority of cancer cells does not lead to a cure. The apparent similarity between cancer cells and stem cells – namely, the ability of the former to grow uncontrollably and that of the latter to continuously renew themselves – has led to the notion that cancers are diseases of stem cells. It is believed that only a fraction of the cells within the tumor mass is capable of generating tumors. Since stem cells have limitless capability to self-renew and generate large numbers of progeny, a few remaining cancer stem cells after treatment may be sufficient to form new tumors and, consequently, cause relapse and prevent cure.

Breast cancer, like other cancers, is traditionally viewed as a disease of cell-cycle control, which determines whether and how fast cells proliferate, and tumor suppressor genes like p53 inhibit tumor initiation by eliminating cells with uncontrolled cell-cycle progression. Recent findings from our laboratory indicate that stem-cell numbers are strictly controlled in tissues and that p53 play a critical role in such homeostasis. We have termed this mechanism “stem-cell homeostasis” and postulated that its defects – the inability to eliminate overproduced stem cells – contribute to tumor initiation and progression. In particular, we have found that stem cells have to divide slower than normal in the absence of p53, which raises an intriguing possibility that cancer may also arise from an inability to slow down from the normal pace of cell division. In other words, our findings suggest that, in some cancer stem cells at least, there is no acceleration of cell-cycle progression and, consequently, such cancers may not be effectively treated using drugs that target the cellular machinery responsible for excessive cell growth, which is the conventional approach, since stem cells in these cancers behave like normal cells.

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In this application, we want to explore novel ways to identify predictors of breast cancer treatment and prognosis and to eliminate the ability of breast cancer stem cells to renew themselves. Asymmetric cell division is a process by which a cell divides to generate two different daughter cells. Such divisions allow stem cells to balance the competing needs of self-renewal and differentiation by producing a daughter that remains as a stem cell (self-renewal) and another that becomes a mature cell (differentiation). We demonstrated recently that in mice, Numb and Numbl proteins allow neural stem cells to balance self-renewal and differentiation by segregating primarily into the stem-cell daughter to promote its fate. We show that eliminating Numb and Numbl, or their partner ACBD3, abolishes the self-renewing capability of neural stem cells. Conversely, forcing the proteins to be active in both daughter cells forces both to choose self-renewal over differentiation, which in turn triggers the mechanism that controls stem-cell numbers. The proposed research has two aims. In one set of experiments, we will manipulate Numb-ACBD3 activity in mammary glands to trigger stem-cell homeostasis and identify genes affected by such changes. We will examine whether the identified genes are markers and regulators of breast cancer stem cells and whether they can serve as predictors of breast cancer treatment and prognosis. Another set of experiments will manipulate Numb-ACBD3 activity in a mouse model of breast cancer to examine whether cancer stem cells can be forced to undergo divisions that eliminates themselves.

Our proposal addresses two fundamental issues in cancer biology and treatment – namely, a lack of signatures that can guide cancer treatment and predict outcome and why killing the vast majority of cancer cells does not lead to a cure. We propose that some cancer stem cells may not be effectively eliminated by targeting the machinery for excessive cell proliferation, the conventional approach. By testing a novel approach to manipulate normal and cancer stem cells, our work has a high probability of yielding markers that can predict chemotherapy efficacy and breast cancer prognosis within the 3-year funding period. In the long term, it is also quite possible that such markers and regulators can be used as baits for identifying novel therapeutic agents for treating breast cancer.

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PI Name: Yun-Ling Zheng, PhD

Institution: Georgetown University, Lombardi Comprehensive Cancer Center

Mechanism: Investigator Initiated Research

Application Title: Chromosome arm-specific telomere lengths and breast cancer risk

Abstract

Breast cancer is the most common cancer in women. Although hormonal and reproductive factors, such as pregnancy history and age at menopause, are well documented contributors to breast cancer risk, there is also strong evidence for a genetic component. The discovery that mutations in the breast cancer 1 (BRCA1) and 2 (BRCA2) genes increase the risk of breast cancer has radically transformed our understanding of its genetic basis, leading to improved management for women with a family history of breast cancer. However, the genetic risk factors for breast cancer in the general population (sporadic cases) are less well understood. Recent efforts by studies that searched whole genome for DNA sequence variations have discovered at least eleven common genetic variants for sporadic breast cancer. These recent discoveries are shedding light on important mechanisms in breast cancer development. However, these common genetic variants are unlikely to be very useful for breast cancer risk assessment in individual women because of their weak associations with risk.

With an estimated 1,152,161 new cases diagnosed worldwide per year, new prevention strategies are needed to further reduce the incidence of this disease. A complete understanding of the risk factors for breast cancer is the critical key for an effective prevention. Research regarding chromosomal telomeres (DNA sequences that cap the end of each chromosome arm) is very promising for this goal because abnormal telomeres (too short or too long) are a common feature in breast tumors. However, the relationship between telomere lengths of specific chromosomal arms and breast cancer risk is currently unknown, but critically important since the genetic abnormalities leading to breast cancer are not random but are concentrated in specific regions of certain chromosomal arms. In this proposed study, we seek to test the hypothesis that optimal telomere length is required for each chromosome arm to maintain its normal function and stability. Thus, women who have “non-optimal” telomere length on certain chromosome arms are susceptible to accumulating specific genetic abnormalities that will increase their risk of breast cancer.

This study will identify and evaluate chromosome arm-specific telomere lengths as novel measures of breast cancer risk. The specific aims are to: (i) conduct a genome-wide telomere association study to identify which chromosome arm-specific telomeres are significantly associated with breast cancer risk; and (ii) develop a telomere length profile as a novel tool for breast cancer risk assessment. Using a case-control study design, the chromosome arm-specific telomere lengths on 46 human chromosomes will be measured by telomere quantitative fluorescent in situ hybridization using white blood cells. Our preliminary data suggest that the telomere lengths on short arms of chromosomes 9 and X are strongly linked to breast cancer risk in sporadic cases. This study is designed to extend these promising preliminary findings and to identify additional arm-specific telomeres that are linked to breast cancer risk. If confirmed, chromosome arm-specific telomere lengths will

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be recognized as a new class of risk factors and could be used as a set of novel biomarkers in conjunction with current prediction models to significantly enhance breast cancer risk prediction for an individual woman without family history of breast cancer.

Results from this study can have a broad impact on both health care and basic science. A personalized assessment of breast cancer risk will allow women at truly elevated risks (based on chromosome telomere analysis) to make informed decisions regarding the frequency and method of future screening and the appropriateness of breast cancer prevention strategies, while women with no elevated risk may gain peace of mind. The focus of image-based screening on women at truly high risk can result in earlier detection that leads to less aggressive treatment and increased survival. Hence, accurate assessment of breast cancer risk has the potential to greatly improve both quality of life (potentially obviating the need for aggressive chemotherapy) and life expectancy for these women. An assessment of breast-cancer risk based on telomere analysis is highly desirable as telomere length is thought to be modifiable with life style change, i.e., reducing life stress or increasing physical activity, or with chemical treatment. Understanding the link between arm-specific telomeres and breast cancer risk will focus cancer research to search for drugable molecules to enhance telomere health for cancer prevention. It is reasonable to expect that in the future drugs will be available to enhance telomere health and women with knowledge of their telomere length profile will have the option of a new class of preventive treatment.

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PI Name: Eli Gilboa, PhD

Institution: University of Miami School of Medicine

Mechanism: Investigator Initiated Research

Application Title: Potentiating Tumor immunity in breast cancer patients using aptamer-targeted Foxp3 siRNA to inactivate regulatory T cells

Abstract

Immune-based therapy is emerging as a promising modality to control tumor progression in cancer, including breast cancer. A main focus is to develop cancer vaccines which are designed to elicit immune responses to reverse cancer progression. Several promising vaccination strategies are currently undergoing clinical testing. It is, however, becoming clear that the effectiveness of cancer vaccines is compromised because tumors have evolved strategies to protect themselves from the otherwise effective antitumor immune response elicited by the vaccines. Hence, developing treatments which mitigate such immune suppressive strategies will be paramount to control cancer progression. There is also broad consensus that a major mechanism of immune suppression in cancer is mediated by regulatory T cells. Arguably, eliminating or inactivating such regulatory T cells – the focus of this proposal – will contribute to successful immune control of cancer, more than was previously thought.

Current strategies to eliminate regulatory T cells in cancer patients employ antibodies or protein-based agents which target a product expressed preferentially on the regulatory T cells called CD25. Yet, the development of effective clinical protocols is hindered for two main reasons. One reason is that CD25 is not specific to regulatory T cells which gives rise to complications and potential toxicity. Second, access to protein-based therapeutic agents is limited and uncertain because the development of clinical grade proteins is expensive (because proteins are made in cells) to a point that only companies have the wherewithal to generate such reagents which they provide to the academic community on a highly restricted manner. Thus, despite promising observations from murine preclinical tumor models, the use of antibodies in clinical settings is limited.

In the project proposed in this application we will use a novel platform technology to develop a therapeutic agent capable of inactivating the immune suppressive regulatory T cells in cancer patients, including but not limited to patients with breast cancer. There are two unique features to this therapeutic agent which addresses the limitations of current strategies. First, the reagent is composed of an oligonucleotide which, unlike proteins, can be synthesized in a cell-free chemical process which is significantly simpler and less expensive. Second the oligonucleotide-based drug is targeted to the appropriate cells in the treated patient, the regulatory T cells. Targeting will require much less drug to be used which will significantly reduce cost and risk of toxicity.

The central hypothesis of this proposal is that oligonucleotide based inhibitors can inactivate Treg, enhance tumor immunity, and provide clinical benefit to patients with breast cancer. In the study proposed in this application we will develop such oligonucleotide-based reagents and evaluate their ability to inactivate regulatory T cells and potentiate vaccine-induced tumor

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immunity in mice using stringent and highly relevant models for breast cancer. The studies in mice will identify best-in-class reagents and provide the guidelines for a phase I clinical trial in patients with breast cancer that will test the safety of administering such reagents to patients and determine the biological dose required to inactivate regulatory T cells.

Accomplishing the goals set forth in this application will provide the guidelines and set the stage for evaluating the clinical benefit of depleting Treg when used in combination with any of several promising vaccination strategies currently under development.

Countering the suppressive action of regulatory T cells in patients with breast cancer will synergize with cancer vaccination protocols and will significantly enhance the clinical benefits to the patients. Notwithstanding the focus of this proposal to breast cancer patients, regulatory T cells represent a major mechanism that many tumors use to protect from the consequence of immune elimination and hence the strategies developed here will have broad applicability to cancer patients.

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PI Name: Cheryl Jorcyk, PhD

Institution: Boise State University

Mechanism: Investigator Initiated Research

Application Title: Analysis of oncostatin M in breast cancer metastasis to bone for the purpose of inhibiting disease progression

Abstract

Breast cancer afflicts millions of women across the world. Most breast cancer is lethal as a result of local invasion and the metastasis of cancer cells from the primary tumor to other tissues. Tumors metastasize by a process that involves the spread of cancer cells through the circulatory or lymph system to establish secondary (metastatic) tumors. Approximately 80% of patients with metastatic breast cancer develop bone metastases, while liver or lung metastases affect approximately 25% of these patients. It is the metastases to the bone that produce serious pain, fractures, bone deformity, hypercalcemia, and spinal cord compression. Bone metastases can be classified as either osteolytic (bone-resorbing) or osteoblastic (bone-forming). Most bone lesions caused by breast cancer are osteolytic. The vast majority of bone destruction in these lesions is caused by cancer cell-mediated enhanced recruitment and activity of bone cells, or osteoclasts, necessary for bone resorption.

Oncostatin M (OSM) is a protein that is produced and secreted by many cells of the immune system. It binds to its receptors found on appropriate target cells, such as breast cancer cells, and subsequently triggers downstream signals that change how the target cell is functioning. Relative to most immune cell-produced proteins, very little is known about the role of OSM in breast cancer. Our lab and other researchers have recently demonstrated that OSM will induce breast tumor cell detachment, secretion of cellular enzymes needed for degradation of tissue surrounding the cancer cells, and production of factors needed for new blood vessel formation and invasive capacity. These are all hallmarks of an environment conducive for tumor progression and metastasis. In addition, evidence from the literature and our preliminary results suggest that OSM could promote osteoclastogenesis (the formation of osteoclasts), bone resorption, and bone metastases. Our hypothesis is that OSM is important in breast cancer metastasis to the bone. The goal of our proposal is to examine the role OSM plays in metastatic breast cancer and to demonstrate that OSM promotes the formation of bone metastases *in vivo*. Furthermore, in the proposed work we will also test the therapeutic effect of an antibody against OSM's receptor in both preventative and therapeutic studies in a mouse model that mimics breast cancer metastasis to bone in humans.

The results of our work will have several important implications. First, results demonstrating that OSM promotes the formation of bone metastasis will make OSM a target for the development of therapeutics. To date, there has been no attempt at inhibiting OSM expression or function for cancer therapy. Second, a human neutralizing antibody to the beta subunit of the OSM receptor (OSMR?) is currently owned by Genentech (South San Francisco, CA), and based on results from the work proposed here, could quickly be moved into clinical trials. Overall, positive results from our research could rapidly lead to clinical

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testing of an existing antibody and the development of novel and more effective treatments for breast cancer metastasis to the bone within the decade.

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PI Name: Konstantin Christov, MD, PhD

Institution: University of Illinois at Chicago

Mechanism: Investigator Initiated Research

Application Title: Role of a novel RAR-beta-5 isoform in the resistance of breast cancer cells to retinoids

Abstract

Over the last decade, significant progress has been made in the prevention and treatment of ER-positive (ER+) breast cancer by tamoxifen and recently by other agents. These agents are able to suppress the development and growth of about 50% of ER+ breast cancers. Until recently there has been a lack of effective agents that can prevent the development and progression of ER-negative (ER-) breast cancer, which make up about 30% of all breast cancers. One potential alternative for the treatment and prevention of ER- breast cancer are retinoids (vitamin A analogs), which in pre-clinical and clinical studies have shown effectiveness in suppressing both ER+ and ER- breast cancers. New classes of retinoids have recently been developed (called rexinoids), and they are currently being used in various pre-clinical and clinical trials for the prevention and treatment of breast and other cancers.

Most data indicate that retinoids can suppress cancer development by modulating the activity of specific retinoid receptors localized in the nucleus (i.e, the center of the cell). Two groups of retinoid receptors have been identified and each of them consists of 3 different subtypes. Normal breast cells usually express all subtypes of retinoid receptors (RARs a,b,g and RXRs a,b,g), whereas one of them, RAR-beta-2 (RARb2), is lost in most breast carcinomas, suggesting its potential tumor suppressor role. Restoration of RARb2 expression by retinoids and other agents has been considered a promising strategy for increasing tumor cell sensitivity to retinoids and for promising response in clinical trials.

Recently we identified a novel RAR-beta isoform (a kind of protein), RAR-beta-5 (RARb5) that is expressed in normal and tumor breast epithelial cells. It appears that RARb5 tends to get expressed more in ER- breast cancer cell lines, which are resistant to retinoids, therefore patients that have ER- breast cancer may not benefit from clinical trials with retinoids. This is important, because retinoids, when given for a long time, might also have some bad side effects in patients' normal tissues. negative. Thus, a promising strategy to increase sensitivity of breast tumor cells to retinoids would be to lower the amount of RARb5 expression with selective retinoids or with other agents.

We will also examine the feasibility of tumor cells taken from breast carcinomas at the time of surgery and grown in vitro (in a test tube), for studies on retinoid receptors and their modulation by retinoids. These tumor cells are called early passages (EPBC) and are closer in biology to primary cancers than to established breast cancer cell lines and therefore the information about RARb5 expression and its regulation by retinoids in EPBC would be more reliable for clinical implication, than when established breast cancer cell lines have been used.

The data obtained may help in identifying RARb5 as a potential biomarker (i.e., an indication) of malignancy and/or resistance of breast pre-malignant and malignant cells to

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retinoids. Therefore, patients who have breast pre-malignant lesions and carcinomas that express RAR β 5 may not benefit from clinical trials with retinoids.

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PI Name: Seema Khan, MD

Institution: Northwestern University

Mechanism: Investigator Initiated Research

Application Title: Topical transdermal therapy for breast cancer prevention using dendrimer nanoparticles for drug delivery

Abstract

Breast cancer prevention is an essential component of breast cancer eradication. The ideal breast cancer prevention strategy should have high effectiveness and no toxicity. The only proven breast cancer prevention medications are tamoxifen and its close relative, raloxifene, both are taken by mouth (orally). When drugs are taken orally, they reach the breast in sufficient amounts, but they also circulate through the whole body and cause unwanted side-effects. The prevention of breast cancer and the treatment of non-invasive breast cancer require only that the drug concentration in the breast tissue should be high enough for the treatment to be effective, but does not require that the rest of the body be exposed to drug. The long-term, big-picture goal of this collaborative grant application is to develop methods for delivery of anti-tumor medications through the skin of the breast for the treatment of non-invasive breast cancer, and for breast cancer prevention.

Tamoxifen is inactive and requires conversion in the liver and elsewhere, to active forms. The two most important ones are called endoxifen and 4-hydroxytamoxifen (4-OHT). We are soon to start an early phase clinical trial funded by the Division of Cancer Prevention of the National Cancer Institute, testing 4-OHT gel applied to the skin of the breast in women with ductal carcinoma in situ (DCIS), but 4-OHT by itself penetrates poorly through the skin and breast tissue concentrations tend to be low. It is possible that endoxifen will have significant advantages over 4-OHT, both for delivery through the skin, and for effectiveness against breast cancer cells. Our study will be the first to test endoxifen delivery through the skin for breast cancer prevention. We hypothesize that the penetration of drugs through the skin can be substantially improved by combining them with dendrimer nanoparticles (a nano-sized branching structure, 1/10,000 the thickness of human hair) and other agents that improve skin penetration, so that the treatment is distributed throughout the entire breast, and reaches effective concentrations in the target cells, but with much lower circulating levels. In this way, the side effects associated with oral use of these agents can be reduced or eliminated. In addition to reducing the side effects of tamoxifen-related agents, our approach of direct delivery of active drug to the breast has another possible advantage. About one-third of women who take tamoxifen do not benefit from it because tamoxifen is inefficiently converted to the active form in their bodies. This form of tamoxifen resistance can be overcome by delivery of 4-OHT or endoxifen through the breast skin, therefore expanding the number of women who can benefit from preventive therapy.

Our experimental design involves three aims, the first two of which are highly interactive and will occur in parallel. Aim 1: Dr. Khan's group at Northwestern University (NU) will test 4-OHT and endoxifen in the lab for permeation through human skin (harvested from mastectomy specimens); in addition, a whole range of compounds prepared by Dr. Hong (see Aim 2), will be tested. The most successful preparations will be returned to Dr. Hong who will "tweak" them further by adding more drug molecules or adding more permeation enhancers to them. These will be re-tested at NU, in a back-and-forth exchange until we are satisfied that the

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best permeability has been achieved. The “winners” will then be taken through careful experiments in the same skin test system to define the time it takes to get maximal delivery through the skin. Aim 2) Dr. Hong (University of Illinois at Chicago) will design and produce the drug-dendrimer preparations, characterize them, and modify them as needed. Aim 3) The best agents will then be tested against breast cancer cells, first in culture, then as transplanted tumors in mice, to see which is most effective at preventing the growth of transplanted tumors.

At the end of this three-year project, we will have defined the optimal 4-OHT or endoxifen preparations for further testing. After this project is completed, we will need one more set of studies, to test the optimal candidate agents in animal models in which tumors form spontaneously and compare the effectiveness of these optimized tamoxifen derivatives against the standard approach of oral tamoxifen therapy. These future studies will also test the safety of the preparations in advance of an application to the Food and Drug Administration (FDA) for human testing of these complexes. Thus the clinical applicability of this project should be relatively rapid. All women who take tamoxifen by mouth are exposed to its active forms, 4-OHT and endoxifen, so that the effectiveness and side-effects of these are well known. Dendrimers are also approved by FDA for some early-phase trials in topical vaginal application (Vivagel™, Starpharma, Inc.) to prevent sexually transmitted diseases including HIV. Therefore, taking the next step in these studies (to human trials) will be relatively quick. Positive findings in this study will have important ramifications for the topical use of other breast cancer chemopreventive agents whose use as oral agents is not possible at the moment because of concerns about unwanted or dangerous side-effects.

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PI Name: Yin-Yuan Mo, PhD

Institution: Southern Illinois University School of Medicine

Mechanism: Investigator Initiated Research

Application Title: Regulation of estrogen-independent growth by microRNAs in ER positive breast cancer cells

Abstract

It is well known that estrogen plays a critical role in breast cancer development because estrogen can stimulate cancer cell growth through its receptor called estrogen receptor (ER). Thus, specific targeting the estrogen signaling pathway provides an effective way to treat breast cancer because about 70% of breast tumors are ER positive. Several clinical drugs currently available target either estrogen synthesis such as aromatase inhibitors or ER such as tamoxifen. However, over 30% of the ER-positive tumors fail to respond to tamoxifen therapy; moreover, those breast tumors that initially respond to tamoxifen will frequently develop resistance to the treatment. Although it is believed that this is likely due to the activation of estrogen-independent signaling, the precise molecular mechanism is not fully understood. From the clinical standpoint view, this is a significant challenge to physicians to determine the treatment options for ER-positive breast tumors due to the lack of reliable biomarkers for this subpopulation.

In this proposal, we will test our hypothesis that microRNAs play a key role in regulation of those genes required for estrogen-independent growth such that deregulation of these microRNAs may cause resistance to tamoxifen. MicroRNAs are tiny RNA molecules produced in our body, just like many other genes. However, these RNA molecules are very unique and powerful in that 1) they are small and do not produce proteins and 2) they can regulate numerous protein-making genes. In particular, accumulating evidence indicates that microRNAs could play an important role in breast cancer development and progression. Given that microRNAs can regulate a large number of protein-making genes, microRNAs can play a fundamental role in diverse cellular pathways including the estrogen-independent growth. Therefore, in this application we will determine what kinds of microRNAs are important to estrogen-independent growth, which could ultimately lead to tamoxifen resistance. Our preliminary studies have identified that 6 microRNAs are sufficient to confer estrogen-independent growth and tamoxifen resistance. Accordingly, we propose three specific aims to define the minimal number of microRNAs which can confer estrogen-independent growth (Aim 1); and to determine the clinical relevance of the microRNAs associated with estrogen-independent growth by profiling microRNAs in clinical specimens from breast cancer patients who were initially identified to be ER positive but failed to respond to tamoxifen (Aim 2); and dissect molecular mechanisms of microRNA-mediated estrogen-independent growth and tamoxifen resistance (Aim 3). Although several microRNAs have been reported to play a role in tamoxifen resistance by targeting ER, the 6 microRNAs identified in this study have no effect on ER, suggesting a novel mechanism involved. Thus, the proposed study will provide a better understanding of the mechanism underlying the non-responsiveness of the ER-positive tumors to tamoxifen. Moreover, such microRNAs serve as biomarkers to predict the therapeutic response.

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PI Name: Brittney-Shea Herbert, PhD

Institution: Indiana University

Mechanism: Investigator Initiated Research

Application Title: Targeting telomerase for the treatment of refractory breast cancers

Abstract

Breast cancer is the most common cancer among women, and the second leading cause of cancer related deaths. Despite this incidence, mortality has declined due to improved screening and more effective treatments. However, approximately 20% of women with breast cancer will develop metastatic disease which, while treatable, remains largely incurable. Therefore, continued investigations into breast cancer therapy are warranted for improved patient outcome and overall survival. For instance, HER2 amplification in breast cancer is associated with a more aggressive disease, greater likelihood of recurrence, and decreased survival compared to women with HER2-negative breast cancer. Herceptin (trastuzumab) is a monoclonal antibody that inhibits HER2 activity, making this compound an important therapeutic option for patients with HER2-positive breast cancer. However, resistance to trastuzumab develops rapidly in a large number of breast cancer patients. Furthermore, the majority of breast cancer therapies target the bulk tumor cells, but leave intact a certain population of cells which may be drug-resistant. Telomerase is an enzyme found in the majority of cancers compared to benign tumors and plays a major role in unlimited cellular growth and tumorigenesis. Telomerase inhibition results in the erosion of DNA at the ends of chromosomes (called telomeres), leading to cell death or growth arrest. Preclinical data from our laboratory have demonstrated that up-regulation of tumor cell telomerase is an important mechanism for breast cancer growth and its inhibition can lead to reduction in tumor cell growth. Recently, novel findings from our laboratory have demonstrated that telomerase inhibition by a novel class of compounds that specifically target telomerase, leads to sensitization to trastuzumab and enhanced cancer cell killing in trastuzumab-resistant breast cancer cells. Our data on this telomerase inhibitor, called imetelstat, have led to Phase I/II clinical trials for breast cancer as well as other cancers. These findings implicate that telomerase antagonists have potential use in the treatment of cancers that have developed resistance to traditional cancer therapy.

Hypothesis and how it will be tested: This stimulating data has prompted us to investigate the hypothesis that telomerase inhibition will synergize with trastuzumab in patients with HER2+ refractory disease. Using combination therapy to target essential hallmarks of cancer (limitless cell growth and drug resistance), such as with telomerase antagonists and trastuzumab, is novel and hypothesized to have greater effects on reducing recurrence or metastases, than either agent alone. We propose that a solution to metastatic and refractory breast disease will be inclusion of the novel telomerase antagonist agent imetelstat in breast therapeutic regimens in the clinic. We aim to test our hypothesis by investigating the mechanisms of how our telomerase inhibitor functions in drug-resistant breast cancer cells, specifically its effects on HER2 and growth factor signaling pathways. In addition, we aim to provide evidence using animal models that telomerase inhibition can effectively target trastuzumab-resistant breast cancer cell growth and synergize with Herceptin.

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Importance of research and impact on breast cancer patients: The research proposed here aims to answer some fundamental biological questions on the role of telomerase in breast cancer with direct therapeutic applications. Targeting drug-resistant breast cancers with telomerase inhibitors is a novel concept that needs to be tested. If we could demonstrate that our telomerase inhibitor targets rare populations of cells responsible for relapse and metastasis, a whole new therapeutic strategy can be developed for patients with refractory breast cancer. Data obtained in this proposal will be immediately implemented in breast cancer clinical trials, including those at our institution with our co-investigator Dr. Kathy Miller, a breast cancer oncologist who has played a major role in important breast cancer clinical trials. Completion of the proposed research would have important and significant clinical implications for breast cancer patients with metastatic or recurrent/refractory disease to improve their quality and quantity of life with novel molecular targeting.

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PI Name: Rick Rogers, PhD

Institution: Harvard University, School of Public Health

Mechanism: Investigator Initiated Research

Application Title: Proposed primary antiangiogenic therapy - computational modeling support

Abstract

We hypothesize a new method of treatment for early stage breast cancer. Rather than trying to kill cancer cells, the new therapy is designed to prolong remission indefinitely. This project started 12 years ago when we were trying to understand data in a Milan database that showed two peaks in relapse events. There was an early sharp peak at about 18 months past surgery, then a minimum at 4 years, followed by a broad second peak with maximum at 5-6 years and gradually decreasing to at least 15 years.

The long assumed continuous growth model could not explain the bimodal relapse data. We suspected that dormancy might somehow play a role but we had no knowledge of what each peak represented. Both peaks were significant. Fifty to 80% of all relapses occurred in the first peak. Very similar bimodal relapse and associated mortality patterns have now been identified in at least 13 independent data bases from the US, Europe and Asia.

Using computer simulation to explore the meaning of these data, we suggested that temporary dormancy is very common in breast cancer and furthermore the surgery to remove the primary tumor often kick-starts growth of dormant distant micrometastases.

For premenopausal patients with positive lymph nodes there is a very early relapse mode in which surgery induces angiogenesis in patients who have micrometastases waiting for a blood supply. This occurs in 20% of such patients and this percentage increases with the number of positive nodes. This relapse mode seems to aptly describe Susan Komen's disease as described by Nancy Brinker. Susan was diagnosed at age 32 with breast cancer. She relapsed 6 months after mastectomy with sites in lung and lymph nodes and died 2 years later.

If our conjecture is true, the implication uniquely advances our understanding of breast cancer. This quantitatively explains why early detection is more beneficial for women 50-59 than it is for women 40-49. This also at least partly explains the excess mortality of blacks compared to whites. Since the average age of diagnosis of blacks is 46 while for whites it is 57, black breast cancer is mainly premenopausal and white breast cancer is mainly postmenopausal. What about black women who are diagnosed at an older age? It turns out that there is an inversion in that over age 57, black women have less mortality than white women. We are implying that the very early relapses for premenopausal node positive women, the higher benefit of mammographic screening for women 50-59, and the excess mortality of blacks over white are all the same effect but viewed from different perspectives. It is like the parable of the blind men from Hindustan and the elephant.

We propose starting an antiangiogenic therapy before surgery. The therapy would protect against relapse as long as it continues. This requires that the therapy must be nontoxic and

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it cannot interfere with wound healing. What drugs can be used to accomplish this? Answers to this question come from the recent observation that women with Down syndrome do not get breast cancer. Since Down syndrome persons have between 2 and 3 copies of chromosome 21 while normal persons have 2 copies, there are several gene products from that chromosome that might function to prevent a blood supply from forming to nourish a micrometastasis. Two proteins identified are endostatin and the recently reported DSCR1. Endostatin is better known and studied so we will focus on that. Endostatin has been found to be toxicity-free.

The new therapeutic idea is to preoperatively (before any surgery) measure the background level of endostatin in the blood, raise it to a level typical of a person with Down syndrome and keep it at that level indefinitely. Surgery can then be conducted conventionally.

This therapy should solve the single biggest problem with conventional cancer therapy. That is, while conventional drugs initially work fairly well, drug resistance gradually sets in and the therapy no longer helps. Meanwhile, toxicity continues unabated. This apparently won't happen in early stage cancer with the new therapy at least based on the Down syndrome connection.

Of course, this therapy needs to be tested. If it works as predicted, breast cancer will no longer be a major public health concern. It could take a decade to plan and conduct the tests. We are requesting funds to start this process and help design a clinical trial or pilot study and evaluate results.

We propose to develop computer programs to aid in the design of trials and also aid in interpreting results. There are two issues that we plan to address. First, each of us has a distinct level of endostatin which has to be first measured and then adjusted to a Down syndrome level. Second, historically there have been problems in manufacturing endostatin since it is a protein rather than a small molecule as are usual drugs. The bioactivity of endostatin has not been consistent and has varied according to who has been making the drug. We have to plan on the possibility that the manufactured endostatin that will be used might not be as effective as natural endostatin. This has to be accounted for in any plan to test the idea in a trial. We propose doing this using computer simulation.

The therapy we are proposing should reduce overall mortality from breast cancer and the racial disparity in outcome. This research will help new patients but unfortunately not likely help existing patients other than just improving our knowledge of the disease.

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PI Name: Amy Yee, PhD

Institution: Tufts University

Mechanism: Investigator Initiated Research

Application Title: Pre-clinical analysis of a novel combinatorial therapeutic strategy for invasive breast cancer and prevention of recurrence

Abstract

Rationale and Hypothesis. For the breast cancer patient, the clinical objective must be the prevention of recurrence and of fatal metastases. The success of a clinical trial necessarily requires the identification of the tumor venues in which the treatments have the best opportunity to be efficacious. Constitutive Wnt signaling is widely linked to breast and other cancers and to mammary tumor stem cells, a probable source of recurrent cancer. Thus, agents that block Wnt signaling are predicted to have an impact in treating breast cancer, yet few exist. We focus on the HBP1 transcriptional repressor, which inhibits Wnt signaling and proliferation. We published that the HBP1 gene has a unique link to invasive breast cancer: 1) Decreases or mutations of HBP1 were correlated with invasive breast cancer; had profound effects on tumorigenic proliferation and invasiveness; and 2) Decreases in both HBP1 and the Wnt inhibitor SFRP1 lead to an exceptionally poor prognosis with a decreased relapse free survival. Other work has shown that the SFRP1 gene is epigenetically silenced in invasive breast cancer with a poor prognosis. Together, the poor prognosis phenotype of decreased HBP1 and decreased SFRP1 is part of molecular circuitry to amplify Wnt signaling—highlighting the deleterious consequences of excessive Wnt signaling.

We reasoned that elevating both HBP1 and SFRP1 might be a good strategy to block Wnt signaling and prevent a poor-prognosis breast tumor phenotype. For the reasons outlined below, we tested the epigenetics-based chemotherapeutic agent Decitabine and the green-tea compound EGCG. Indeed, our preliminary results highlight that Decitabine and EGCG are synergistic for the inducing SFRP1 and HBP1 to block Wnt signaling and to suppress breast tumorigenesis in a pre-clinical animal model. Decitabine is currently the only epigenetics-based chemotherapeutic in clinical trials and has been FDA-approved for hematological malignancies. While Decitabine has known side-effects (myelosuppression), Decitabine is in Phase 1 trials for solid tumors, but will require collaborative agents to have maximal efficacy. EGCG and green tea are in many phase 1 clinical trials and is well tolerated. Specifically, we have published that EGCG suppresses Wnt signaling and invasiveness through the induction of HBP1, providing a concrete mechanism for blocking Wnt signaling. While neither EGCG nor Decitabine were specific for Wnt signaling, there are no compounds in clinical trials that have been reported to block Wnt signaling and a major advantage is that both EGCG and Decitabine are already in clinical trials.

The overall goals are to develop EGCG and Decitabine as a combination regimen for invasive breast cancer and to use knowledge of Wnt signaling, HBP1, SFRP1 to determine the tumor conditions that may confer susceptibility. Furthermore, we will develop the relatively safe green tea compound EGCG as a collaborative agent to increase efficacy and reduce side effects of Decitabine. In this way, we hope to use molecular parameters to refine existing

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phase 1 drugs for a new application in treating breast cancer. Our studies are configured for the optimal applicability towards an eventual breast cancer clinical trial.

Breast Cancer Impact. Breast cancer relapse and recurrence is the major impediment to improved patient outcomes. For the breast cancer patient, recurrence can occur in 0 years, 5, years, or even 20 years after the initial diagnosis. Thus, there must be better tools and treatments to definitively treat breast cancer to prevent the often-fatal recurrences. A combination strategy with Decitabine and EGCG could have exceptional potential for treating breast cancer and preventing recurrence. Delineation of two genes that specify poor prognosis is an important aspect of the studies. A unique, but practical, aspect is the refinement of gene parameters that may provide insights into which patients might achieve maximal benefit, while limiting unnecessary side effects. The availability of excellent pre-clinical models may further refine the parameters, once clinical trials begin. With our work, we hope to advance knowledge towards treatment strategies aimed at definitive treatment of breast cancer and towards preventing recurrence.

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PI Name: Xiao Zhen Zhou, MD

Institution: Beth Israel Deaconess Medical Center, Boston

Mechanism: Investigator Initiated Research

Application Title: Impact of the telomerase inhibitor PinX1 on the development and/or clinical outcome of breast cancer

Abstract

Study Hypothesis and How It will be Tested

The major hypothesis in this study is that a new gene called PinX1 that we discovered earlier (Zhou and Lu, 2001, Cell 107, 347-359) has a major impact on the development and/or clinical outcome of human breast cancer based on the following supporting evidence.

One unique feature for most cancer cells including up to 93 percent of invasive breast cancer have activation of an enzyme called telomerase. Moreover, inhibition of telomerase has been proposed to be an attractive new idea for anticancer treatment due to its ability to selectively kill cancer cells. However, it is not well understood whether and how telomerase activity itself is regulated and how important such regulation is for cancer development.

We have previously identified PinX1 as the first naturally occurring human protein capable of inhibiting telomerase activity. Importantly, reducing PinX1 protein levels makes cancer cells to grow much faster in mice. Together with the fact that PinX1 is located on a region of human chromosome called 8p23 that frequently undergoes spontaneous loss in many cancers including breast cancer, these results suggest that PinX1 may be silenced in breast cancer and thereby affect breast cancer development and/or clinical outcome.

To obtain the supporting evidence for such novel idea, we have successfully removed the PinX1 gene in mice or mouse cells. Importantly, partial loss of PinX1 increases telomerase activity and causes abnormal chromosome structure, which can be fully prevented by reducing telomerase activity. Furthermore, almost all PinX1 mutant mice spontaneously develop many aggressive tumors that are unusual in mice but common in humans, including breast cancer. In addition, we found that PinX1 expression is significantly reduced in about 90 percent of human breast cancer tissues. Finally, we also identified several genetic changes in the PinX1 gene in human breast cancer tissues, which make PinX1 less effective in inhibiting telomerase activity. These preliminary results suggest that loss of PinX1 by reduced protein expression or genetic mutations PinX1 is likely has major impact on the development and/or clinical outcome of breast cancer.

In this proposal, we will test this exciting and important hypothesis using animal models, human breast cancer tissues and cell lines. Specifically, we will first generate new mutant mouse strains to examine whether removal of the PinX1 gene specifically in breast tissues leads increased susceptibility to breast cancer. Then, we will collaborate with J. Dirk Iglehart (the Director of DF/HCC SPORE in Breast Cancer) and Jenny Driver (an oncologist and epidemiologist) to determine the impact of altered PinX1 expression on the development and/or clinical outcome of breast cancer in humans and to determine the impact of altered PinX1 expression on breast cancer phenotypes in human cell lines. Finally, we will collaborate with Drs. Iglehart and Driver to screen for PINX1 genetic alterations in breast tissues, to determine their impact on the development and/or clinical outcome of

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BCa in humans and to determine the impact of PinX1 mutations on breast cancer phenotypes in human cell lines.

Unique Contribution

These findings would likely have major impact on the diagnosis and treatment of breast cancer. It is likely that PinX1 downregulation and/or genetic changes in humans may be an important risk factor and/or biomarker in developing breast cancer and/or leading to poor clinical outcome. Furthermore, our breast-specific PinX1 mutant mice would be a novel breast cancer mouse model for elucidating molecular mechanisms of breast cancer development and testing anticancer drugs such as telomerase inhibitors. Moreover, breast cancer patients with reduced PinX1 expression and/or genetic changes might be ideal human subjects for clinical trials for testing telomerase inhibitors that are being developed. Therefore, our studies will lead to reduction in breast cancer incidence and/or mortality by elucidating new molecular mechanisms, and identifying a new risk factor and/or biomarker, and facilitating testing and clinical trials of telomerase inhibitors that are being developed.

Importance of the Research to Patients with Breast Cancer

Breast cancer is the most common malignancy and is the second leading cause of cancer mortality in western women, with advanced breast cancer patients facing the major therapeutic challenge. It has become evident that a key mechanism for breast cancer development is due to loss of function of certain genes that are critical for inhibiting breast cancer development. The most well known genes in breast cancer include BRCA1 and BRCA2. However, they only account for about 5% of human breast cancer. Up to 60 percent of sporadic breast cancer have been shown to have genetic changes located on chromosome 8p. Moreover, these genetic changes are also associated with advanced tumor stage and aggressive histology. However, the identification and function of the major tumor suppressor located at this region for breast cancer remain elusive. Our findings that PinX1 is located at this region of the chromosome and its function is reduced in the vast majority of breast cancer tissues suggest that PinX1 is a strong candidate for such elusive major tumor suppressor that have major impact on the development and/or clinical outcome of breast cancer. Therefore, our proposed studies will identify a novel risk factor or biomarker for the development and/or clinical outcome of human breast cancer, eventually leading to earlier diagnosis and better management of breast cancer.

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PI Name: Stuart Martin, PhD

Institution: University of Maryland at Baltimore

Mechanism: Investigator Initiated Research

Application Title: Targeting microtentacles on circulating breast tumor cells to reduce metastasis.

Abstract

BACKGROUND

Circulating tumor cells (CTCs) that enter the bloodstream can travel to distant tissues and survive to recur later as the metastatic tumors that cause patient death. As many as 30% of breast cancer patients with no clinical evidence of metastasis have detectable CTCs that strongly predict poor patient outcome. However, while there is currently great effort being directed toward CTCs as indicators of prognosis, very little is being done to specifically target CTCs therapeutically. Surgery and chemotherapies can increase levels of CTCs dramatically if every tumor cell is not successfully removed or destroyed, since the wound healing that follows provides access for tumor cells to the bloodstream. Developing therapies directed against CTCs may provide opportunities to reduce the survival of CTCs released during treatment of the primary tumor.

Our lab has recently discovered unique "microtentacles" (McTNs) that occur on the surface of detached breast tumor cells (Whipple et al., *Cancer Research* 2008; 68:5678-5688). All human cells are supported by a network of internal filaments, known as the cytoskeleton, and our recent work has determined that McTNs are supported by the same cytoskeletal filaments that promote the binding of CTCs to blood vessel walls. Since large epithelial tumor cells are often fragmented when pushed through narrow capillaries by blood pressure, reducing the ability of CTCs to bind to blood vessel walls provides an opportunity to destroy tumor cells by forcing them through capillaries. Nearly 85% of human breast cancers arise as epithelial carcinomas and could be targeted by this mechanism. There are currently numerous studies using cancer cells that are attached to flattened or 3-dimensional extracellular matrix proteins, but extremely few focused on the behavior of tumor cells that are detached and circulating. Given our unique findings with microtentacles, we have been concentrating on identifying the specific proteins that support McTN structure and their ability to promote tumor cell attachment to blood vessel walls.

HYPOTHESIS

We have identified 3 critical cytoskeletal regulators of McTNs (tubulin detyrosination, kinesin motor proteins and tau), and this research plan will test the hypothesis that targeting these mechanisms will reduce the reattachment of CTCs and cause their fragmentation in lung capillaries. This hypothesis will be tested with the following specific aims, using lead compounds that we have already identified to target each of the 3 independent mechanisms. Each aim will test the effect of the lead compounds on CTC metastasis and examine the specific cytoskeletal mechanism in greater detail to further refine the therapeutic target.

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SPECIFIC AIMS

1) Define the role of tubulin detyrosination during McTN generation and metastasis. McTNs are composed of a specific form of tubulin that has lost a tyrosine amino acid at one end. We have identified a lead compound, Parthenolide, that inhibits McTNs by reducing tubulin detyrosination. This will allow us to target McTNs more specifically than existing chemotherapies that affect all cellular tubulin. The effect of Parthenolide on CTCs and the fine structure of McTNs will be investigated and compared to the FDA-approved compounds Colchicine and Taxol.

2) Examine the contribution of kinesin motor proteins to McTN extension, motility and function. Kinesin proteins are motors that pull together detyrosinated tubulin and a more stable cytoskeletal filament called vimentin. Our current work shows that kinesin activity is critical for McTNs and can be targeted with the lead compounds, Tetracaine and Lidocaine. These compounds are already used as local anesthetics and interestingly were shown many years ago to inhibit metastasis, although the mechanism responsible was never defined. We will dynamically measure the effects of these anesthetics on CTCs circulating in living mice to examine whether their effect on McTNs is responsible for their ability to inhibit metastasis. Since there are currently 41 known human kinesin proteins, we will also define which kinesins most strongly regulate McTNs.

3) Target expression of the microtubule-binding protein, tau, to reduce McTNs. The tubulin-stabilizing protein, tau, provides a genetic mechanism that tumor cells can exploit to increase McTNs long-term. We have found that tau is upregulated in 52% of metastatic breast tumors, indicating that it could be a common mechanism for increasing metastasis. Lead compounds are already available to decrease Tau expression, since it is a therapeutic target for Alzheimer's disease. We will test the effect of these two lead compounds on McTNs and metastasis.

ADVANCES

Our work tests new approaches to inhibit metastasis by targeting the cytoskeleton of detached and circulating tumor cells (CTCs), which is currently a significant gap in breast cancer research. We have identified novel microtentacles that are supported by mechanisms that are known to enhance the adhesion of CTCs to blood vessel walls in distant tissues. Since surgery and chemotherapy of primary tumors can greatly increase CTCs, we will test lead compounds against 3 novel mechanisms to reduce adhesion of CTCs to blood vessel walls by targeting McTNs. In each aim, we will test compounds that have already been approved by the FDA for other conditions, which will allow us to more rapidly move to clinical trials in future studies. We aim to identify compounds that could be given in advance of surgical or chemotherapeutic treatment of the primary tumor to reduce the metastatic success of CTCs that escape during treatment of the primary tumor.

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PI Name: Dmitri Artemov, PhD

Institution: Johns Hopkins University, School of Medicine

Mechanism: Investigator Initiated Research

Application Title: Novel two-component delivery system based on Her-2/neu receptor internalization strategy

Abstract

Herceptin, a monoclonal antibody that specifically binds Her-2 receptors that are often found in aggressive breast cancer, is an important component of chemotherapy for Her-2 positive breast cancer. Unfortunately, some tumors that initially respond to this therapy can eventually become resistant to Herceptin and therefore treatment efficiency significantly reduces. In this proposal we will synthesize and test a compound that simultaneously provides specific MR imaging and delivers the cytotoxic drug, Taxol, to Her-2 expressing breast cancer cells. This approach provides (i) noninvasive detection of the susceptible breast cancer cells, (ii) visualizes cell uptake of the compound, and (iii) specifically kills Her-2 positive cells including those that became resistant to Herceptin therapy.

The main innovation of this approach is the two-step targeting of breast cancer cells with a two-component agent. The first antibody-based component specifically recognizes Her-2 receptors and binds to Her-2 positive breast cancer cells. The second component will bind with high specificity to the first component attached to the cancer cell surface and will induce rapid internalization of the entire complex by the cell. A polymer molecule of the second component will carry a combination of MR contrast agent and anticancer drug to the target cancer cell. The high selectivity of this strategy arises from two highly specific steps involved in the internalization process.

Preclinical studies will be performed in cultured human cells and in animal models of human breast cancer. Special emphasis will be made on designing the system to be readily clinically translatable, by utilizing non-toxic and non-immunogenic components, and through extensive testing the approach in vivo in animal models.

This novel strategy for specific and efficient targeting of Her-2/neu positive breast cancer will provide highly selective image-guided delivery of therapy to the target breast cancer cells. Noninvasive MR imaging will provide initial evaluation of the Her-2 availability in the tumor, and will monitor uptake of therapeutic complexes by Her-2 expressing breast cancer cells. Additionally, Her-2 expression can be repetitively measured during the course of the treatment by noninvasive MR imaging. Overall, the new delivery system will provide a unique platform for specific image-guided therapy of Her-2 positive breast cancer that will eventually result in reduced mortality in breast cancer patients.

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PI Name: Neb Duric, PhD

Institution: Karmanos Cancer Institute

Mechanism: Investigator Initiated Research

Application Title: Development of a predictive model for improved, cost-effective breast cancer detection based on biomechanical properties of tissue

Abstract

Mammography has reduced significantly the mortality rate from breast cancer. However, in women with dense breast tissue, the performance of mammography is poor leading to missed cancers. Yet, women with dense breast tissue have an unusually high risk of developing breast cancer. Thus, the performance of mammography is at its worst for women who are at very high risk for developing breast cancer. Consequently, the ability to detect cancers at an early stage, when they are the most curable, is compromised by mammography's low sensitivity in this high-risk group. Patient concerns about the discomfort and radiation that accompanies mammography have also contributed to lower participation rates in breast cancer screening. Furthermore, the radiation that accompanies mammography severely limits its use in younger women, including those that might be at high risk.

The need to shield the radiation, and the associated expense and regulatory requirements, contributes to limited access by restricting installation to specialized centers and hospitals and requiring highly trained staff. Magnetic resonance imaging (MRI) can significantly improve on these limitations by virtue of its relatively comfortable, radiation-free imaging capability. Studies have shown that MRI can impact a large swath of the breast management continuum ranging from risk assessment to diagnosis and treatment monitoring. However, MRI has long been prohibitively expensive for routine use and even less accessible than mammography because of the high cost of installation and maintenance. Therefore, a low-cost alternative to MRI would remove a barrier to reducing breast cancer mortality and morbidity since there is currently a trade-off between the cost effectiveness of mammography and the comfort and imaging performance of MRI. Our goal is to develop techniques, based on a concept known as ultrasound tomography (UST), to eliminate this trade-off and thereby improve performance for women with dense breasts, increase access to breast imaging and improve participation rates. UST is a method that can measure differences in tissue stiffness which can be exploited to support cancer detection.

Conventional reflection ultrasound provide anatomical images (shapes) of breast tumors. However, reflection is just one type of ultrasound signature associated with the acoustic properties of tissue. UST measures other acoustic properties in order to paint a more complete picture of the properties of breast tissue. Our implementation of UST also enables imaging that uses a acoustic dyes which can enhance UST's sensitivity to tumors and provide additional information about them. Consequently, UST has the potential to accurately differentiate cancer from normal tissue and benign disease. Furthermore, UST offers comfort, ease-of-use and low-installation and maintenance costs. In this proposal, we explore the potential of UST imaging in improving breast cancer detection and access to breast cancer screening.

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Our long-term goal is to improve breast cancer survival rates and decrease unnecessary biopsies through a better understanding and measurement of the acoustic properties of cancer. The objective of this application, the next step in pursuit of that goal, is to develop and test the ability of the UST method to detect breast cancer. Our central hypothesis is that acoustic properties of tissue provide a basis for enhanced detection and differentiation of cancer. Our hypothesis has been formulated on the basis of strong preliminary data, from patient studies recently completed in our breast center and will be tested through experiments designed to test the accuracy of the UST method. With the feasibility of UST established by our work and that of other groups, the rationale for the proposed study is that validation of the technique under realistic trial conditions will demonstrate clinical utility, ultimately leading to a practical, low-cost technique for breast cancer detection and characterization. Such an accomplishment would improve breast cancer survival rates by (i) detecting breast cancer at an earlier stage, when it is the most curable and (ii) increasing screening participation rates by offering a comfortable imaging method that can be fielded in almost any community setting.

Building on our supportive preliminary data, we have assembled a diverse research team with the scope and breadth of expertise needed to successfully carry out the proposed work under optimal clinical and research environments. The Karmanos Cancer Institute (KCI) provides a dedicated clinical space, as well as integrated imaging and statistical resources which will provide strong support for the study. Furthermore, the group at KCI, invented and developed the clinical prototype that will be used to support this study. We expect to achieve our objective by pursuing the following specific aims.

Aim 1: Develop and test the UST method without acoustic dyes

Aim 2: Develop and test the UST method with acoustic dyes.

Aim 3: Determine accuracy of the method.

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PI Name: Scott Kaufmann, MD, PhD

Institution: Mayo Clinic and Foundation, Rochester

Mechanism: Investigator Initiated Research

Application Title: Chfr and drug sensitivity of human breast cancer

Abstract

Although drugs such as paclitaxel (Taxol®) and docetaxel (Taxotere®), collectively referred to as taxanes, are often used to treat breast cancer, these agents are effective in only a subset of breast cancer patients. Improved understanding of the biological properties that render some breast cancers sensitive to these agents and others resistant would allow the administration of taxanes only to patients whose tumors have a higher likelihood of responding. This approach to the individualization of breast cancer therapy could potentially avoid the side effects of taxane therapy in patients whose tumors have a low likelihood of responding. Building on our earlier studies examining the manner in which taxanes kill breast cancer cells, we have more recently explored the biology of a protein called Chfr (Checkpoint protein with forkhead-associated and RING domains). Chfr regulates the stability of two enzymes, Aurora A and Plk1, that help cells enter mitosis, the phase of the cell cycle in which taxanes are most toxic. Loss of Chfr expression in other cell types results in increased expression of these enzymes and increased taxane sensitivity. Previous studies have also shown that Chfr expression is diminished or absent in ~40% of clinical breast cancers, although the implications of this observation for response of breast cancers to therapy are unknown. In preliminary studies, we have demonstrated that Chfr downregulation increases paclitaxel sensitivity in breast cancer cell lines independent of its effects on Aurora A and Plk1. These observations suggest that Chfr is altering taxane sensitivity through its effects on some other protein. In further studies, we have demonstrated a striking correlation between Chfr expression and clinical outcome: Patients with invasive breast cancer that exhibits absent or low (0 or 1+) Chfr staining at the time of diagnosis are 4.5-fold more likely (95% confidence interval 1.5- to 13.2-fold, $p = 0.0008$) to live 7 years without recurrence than patients with tumors that exhibit moderate or strong (2+ or 3+) staining. These preliminary results lead to the HYPOTHESIS that low Chfr expression is a marker of increased sensitivity of human breast cancers to drugs that interfere with mitosis. This hypothesis leads to several PREDICTIONS. First, low Chfr expression might indicate sensitivity to other classes of drugs that target cells in mitosis, not just to taxanes. Second, because Chfr regulates both the stability of other proteins and taxane sensitivity, it should be possible to identify the Chfr-associated protein that is more directly responsible for regulating taxane sensitivity of breast cancer cells; and this Chfr-regulated protein might be an even better marker of taxane sensitivity. Third, if the association between diminished Chfr expression and better clinical outcome demonstrated in our pilot study is more than a chance occurrence, we should be able to replicate the result in an independent group of patients who receive taxane-containing therapy for breast cancer. This replication, which is a necessary step toward determining whether Chfr is a useful predictor of response in the clinic, will also give us the opportunity to determine whether Chfr staining provides any additional information beyond that provided by assays for estrogen receptor expression, Her2 amplification and gene pattern (MammaPrint low risk vs. high risk profile). We propose to test these predictions through the completion of three

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SPECIFIC AIMS. In AIM 1 we will assess the effect of Chfr downregulation on sensitivity of breast cancer cell lines to other agents that act during mitosis, including the epothilone ixabepilone, the Aurora A inhibitor MK-8745, the Plk1 inhibitor BI-2536, and the Eg5 inhibitor AZD 4877. While it is known that low Chfr expression is associated with increased sensitivity to paclitaxel, the impact of Chfr on these other agents, which are examples of classes of drugs currently being tested or considered for testing in breast cancer patients, is unknown. In AIM 2 we will determine how low Chfr expression results in increased paclitaxel sensitivity of breast cancer cells by identifying one or more Chfr-regulated proteins that alter the response to taxanes. By learning how Chfr downregulation sensitizes breast cancer lines to paclitaxel, we hope to develop a better understanding of factors that determine whether breast cancers will respond to taxanes or not. In AIM 3 we will examine the relationship between Chfr expression and response in patients who received taxane-containing therapy for breast cancer. We have chosen to examine pretreatment samples from patients with locally advanced (stage III) breast cancer enrolled in the multi-institution I-SPY Trial because we will be able to correlate Chfr expression specifically with response to taxane therapy [assessed by magnetic resonance imaging (MRI) prior to and after taxane treatment]. In addition, because the tumors have been extensively profiled, we will be able to determine whether Chfr staining adds any new information to existing assays. Collectively, these aims are designed improve our understanding of biological properties that determine whether breast cancer will respond to taxanes or not. If successful, these experiments will contribute to the development of Chfr (or one of the proteins it regulates) as a biomarker of taxane sensitivity, ultimately allowing paclitaxel or docetaxel to be administered only to patients whose tumors have changes that predict a high likelihood of responding to this class of agents.

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PI Name: Keith Knutson, PhD

Institution: Mayo Clinic and Foundation, Rochester

Mechanism: Investigator Initiated Research

Application Title: The role of the immune response in the clinical efficacy of combination trastuzumab and chemotherapy

Abstract

Trastuzumab (Herceptin), an antibody against the HER2 protein, has revolutionized the treatment for these cancers, particularly when combined with chemotherapy. Yet, not all HER2-positive patients respond to trastuzumab/chemotherapy. Additional tissue and blood tests that can better predict the response to trastuzumab are needed to more effectively personalize therapy. Furthermore, additional research into understanding how the combination approach works will lead to further improvements. The general hypothesis addressed in this grant proposal is that trastuzumab, when combined with chemotherapy, actively immunizes patients, like a vaccine, leading to the generation of tumor specific T cells or antibodies that are associated with tumor regression. The funds from this grant will be used to support studies that will examine the immunologic response in both adjuvant and metastatic breast cancer patients treated with trastuzumab. Our specific aims are to: 1) To determine whether anti-HER2 antibody responses, generated during chemotherapy and trastuzumab in breast cancer patients, are associated with clinical responses. We will perform retrospective analyses of endogenous HER2-specific antibody responses using serum samples collected from metastatic breast cancer patients treated with chemotherapy and trastuzumab; and 2) To determine whether a HER2-specific T cell immune response, induced in HER2+ breast cancer patients treated with combination trastuzumab and chemotherapy, is associated with clinical responses to therapy. We will perform a study evaluating T cell and antibody immunity in adjuvant and metastatic breast cancer patients treated with chemotherapy and trastuzumab. We will determine if patients with immunity have better survival and tumor regression. Successful results will help identify those patients most likely to benefit from trastuzumab and provide new avenues for combining trastuzumab/chemotherapy with other immune modulators for better outcomes.

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PI Name: Jonathan Serody, MD

Institution: University of North Carolina at Chapel Hill

Mechanism: Investigator Initiated Research

Application Title: Combined modality therapies for the treatment of metastatic breast cancer

Abstract

Currently, individuals who have breast cancer that has spread outside the breast and tissues around the breast cannot be cured using the available therapies which would include drug or radiation therapy. Our group over the past 10 years has sought new avenues to treat individuals with disseminated breast cancer (metastatic disease). Most recently, we have focused on using the patient's immune system combined with drug therapies to attempt to control the spread of metastatic breast cancer. Previously, we made tumor vaccines using parts of the HER-2/neu protein, that is important in causing cancer in approximately 25% of patients with breast cancer, to produce the immune response. These vaccines were safe for patients, but when given alone were not able to cause slowing of tumor growth or an immune response in the majority of treated patients. Part of the problem was that it took 3-4 months for the vaccine to work and often patients had progression of their cancer before the immune response did occur.

Thus, we went back to the laboratory to come up with a better approach for tumor vaccination and methods to combine this with other therapies typically given in the treatment of patients with metastatic breast cancer. We found that giving the vaccine with trastuzumab (Herceptin™) improved the activity of the vaccine in causing tumor regression in animal models. Additionally, we demonstrated that the chemotherapy drug, vinorelbine, given before or after vaccination did not hurt the ability of animals to respond to the vaccine.

As a result of these findings, we initiated a clinical trial in 2006 supported by an NIH R21 grant that combined vinorelbine, trastuzumab (Herceptin™) and a vaccine using patients' cells coupled with a part of the HER-2/neu protein. While this approach addressed some of the problems with our initial vaccine strategy, we were unable to generate a robust immune response using this vaccine. Previously, we had found that how the vaccine is made is critical in whether the vaccine works. The vaccine that we used previously was not made using the best way to "mature" the vaccine. Thus, we altered the vaccine by incubating it with proteins that increased its function and adding a strategy to get around tumors becoming resistant to immune killing. This new vaccine approach combined with vinorelbine and trastuzumab (Herceptin™) has been given to eight patients. Six of these have completed six cycles of the protocol and of these six, four have had shrinkage of their tumors and perhaps as importantly all four have had immune responses that are 10x greater than that found using our previous vaccine strategies.

The current proposal seeks to provide support to allow us to treat 20 additional patients on the current more active vaccine trial. Additionally, by being able to compare the outcome and vaccine response of this group of 28 patients with the 18 patients treated similarly using an inferior vaccine, we can determine the role of the vaccine in the clinical response of

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patients with metastatic breast cancer and perhaps whether this strategy can improve survival.

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PI Name: Alexei Kisselev, PhD

Institution: Dartmouth Medical School

Mechanism: Investigator Initiated Research

Application Title: Site-specific proteasome inhibitors for the treatment of triple-negative breast cancers

Abstract

Triple-negative breast cancers—TNBCs, i.e., cancers that do not express estrogen receptor (ER), progesterone receptor (PgR), or human epidermal growth factor receptor 2 (HER2)—comprise 15% of all breast cancers but account for 25% of deaths from breast cancer. They are characterized by early age of onset, short time to relapse, and aggressive growth. Treatment agents commonly used for breast cancer, such as tamoxifen, aromatase inhibitors, and Herceptin, are not effective against TNBCs.

Many of genetic mutations in cancer cells impair the tumor cells' ability to repair their own DNA. This vulnerability is exploited for cancer treatment as most chemotherapeutic regimens for cancer include drugs that inflict damage on cellular DNA (termed "DNA-damaging agents"). Normal cells can repair damage from these drugs, but cancer cells are unable to do so because of these mutations and are more likely to die.

TNBC tumors have more mutations than other breast tumors, making them more susceptible to DNA-damaging agents. Thus, initially, TNBCs respond to these compounds better than other tumors. Eventually, however, the tumors return, most likely due to incomplete killing during initial treatment. New drugs are needed to prevent this relapse. We propose that the drugs called proteasome inhibitors are excellent candidates for this role. In yeast cells, the combination of non-lethal mutation of proteasome genes and genes that encode proteins involved in DNA repair makes the cells extremely sensitive to DNA-damaging agents such as those used for breast cancer treatment. We hypothesize that clinically achievable concentrations of proteasome inhibitors—drugs that temporarily shut off aspects of the proteasome's operation—should also sensitize TNBC to DNA-damaging chemotherapeutic agents.

The proteasome is the major protein-recycling machine of the cell: it destroys damaged and no-longer needed proteins. Cancer cells absolutely require this process for their growth and division, and die if it is blocked. Normal body cells are much less sensitive to such inhibition. The proteasome inhibitor bortezomib (Velcade®) is now used for the treatment of multiple myeloma, a cancer of bone marrow. Three more proteasome inhibitors are at different stages of clinical trials. It is likely that the role of proteasomes in DNA repair is to open access for the DNA repair machinery to the damage site by destroying proteins bound to the DNA. Proteasome inhibitors prevent proteasomes from doing this and thus block DNA repair. Velcade's potential as a treatment for breast cancer has not been fully explored. Although it has not shown any clinical benefit for patients with metastatic breast cancer when used as a single agent (not in combination with any other drug), recent data from our laboratory indicate a possible explanation for this failure. Initial trials of Velcade were not targeted to specific subtypes of breast cancer, and the majority of recruited patients had the ER-positive subtype. Working with cells grown in culture, we find that most cells derived from ER-positive breast cancers are resistant to Velcade. Cells derived from TNBC cancers, in contrast, are very sensitive to Velcade, though to kill these cells, stronger inhibition of the proteasome is

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needed than is achievable in patients (because toxic amounts of the drug would be needed). However, clinically-achievable sub-toxic proteasome inhibition should be sufficient to sensitize TNBC cancer cells to DNA-damaging agents. In combination, two such agents should be more effective than either can be alone.

Protein degradation by proteasomes can be compared with the action of a food processor with a six-bladed knife except that the proteasome's molecular "blades" are not identical. These blades are called active sites. Each proteasome contains three different pairs of active sites: a $\beta 5$ pair, a $\beta 1$ pair, and a $\beta 2$ pair. Critical review of the scientific literature suggests that if only the $\beta 5$ pair is damaged, TNBC cells will be very sensitive to chemotherapy; likewise if only the $\beta 1$ pair is damaged. Recent data from our laboratory indicate that selectively damaging one active-site pair does not harm normal cells, but completely destroying the $\beta 5$ sites and either of the remaining two pairs is indeed toxic. Velcade co-inhibits both the $\beta 5$ and $\beta 1$ sites, so it is potentially toxic. Carfilzomib is a selective inhibitor of the $\beta 5$ site that has successfully completed phase II trials in myeloma, and appears to be less toxic than Velcade. We have developed a selective inhibitor of the $\beta 1$ sites that is not toxic to normal cells and are keen to test its capacity to sensitize TNBC cells to DNA-damaging agents.

In short, we hypothesize that site-specific proteasome inhibitors should sensitize TNBC to DNA-damaging drugs. The specific aims of this proposal are as follows:

1. Demonstrate that Velcade and the $\beta 5$ -specific proteasome inhibitor carfilzomib sensitize cells derived from TNBCs to three DNA-damaging drugs, namely doxorubicin, cyclophosphamide (standard pre-surgical and post-surgical treatment of newly diagnosed disease), and a combination of carboplatin and a inhibitor of poly ADP-ribose-polymerase (the latter being a highly promising experimental treatment for a metastatic disease).
2. Test whether the $\beta 1$ -specific proteasome inhibitor developed in our laboratory sensitizes TNBC cells to the same agents.
3. Validate finding of Aims 1 and 2 in animal models of TNBCs.

If our hypothesis correct, we will be in a position to start a phase I clinical trial of proteasome inhibitor-containing combinations in patients with TNBCs within a year after accomplishing these Aims.

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PI Name: James Holland, MD

Institution: Mount Sinai School of Medicine

Mechanism: Investigator Initiated Research

Application Title: Human mammary tumor virus (HMTV) and breast cancer

Abstract

The cause of most human breast cancers (BC) is unknown. BC in mice is caused by the mouse mammary tumor virus (MMTV). On the possibility that human BC might be caused by a closely related virus, we began a search. We found a sequence of the MMTV envelope that is absolutely unique. It does not occur in any other virus nor in human DNA. Using a precise chemical technique, we found this unique identifier in 40% of human BC but not in the normal tissues nearby. If it were genetically inherited the viral components would be in the DNA of every cell in the body; hence we concluded that women whose BC harbor the virus did not inherit it, but got infected by it. We have named this the human mammary tumor virus (HMTV). Subsequently we have identified all the components of the entire virus which is 9,900 nucleic acid bases long. We have shown that it gets incorporated into the DNA of infected cells and have shown exactly where by analyzing the flanking sequences. We have made pictures of HMTV, having multiplied in infected cells, budding off the cell membranes. Several laboratories on three continents have confirmed our findings. We have recently shown that the virus from BC cells can infect normal breast cells and normal white blood cells when they are cultured together.

HMTV is not distributed evenly around the world. In the USA, Mexico, Brazil, Argentina, and Italy where the common house mouse, *mus domesticus* is the indigenous species known to carry MMTV, 30-40% of BC contain the unique viral sequence. In China and Japan where BC is much less common the mouse species is different, MMTV is less pervasive, and only 10% and 12% of BC contain the sequence.

We believe HMTV is a mutant of MMTV. It might exist as a rare component of MMTV populations, particularly virulent for humans. Or it might be a mutation that occurs in MMTV as it maneuvers to infect human cells. To establish that HMTV is a cause of BC we must show that women get infected before the BC develops. To do this we will compare antibodies to components of HMTV in the stored blood of healthy women some of whom later developed BC with women from the same group who did not develop BC. To be convincing, BC patients must show more frequent antibodies, although not necessarily in higher amount because robust responders may more effectively have suppressed the virus and escaped BC. The second requirement is that we show the breast cells which we infect with HMTV behave like cancer cells in test tube experiments and when transplanted into specially bred mice that are immunodeficient and allow human cancers to grow.

Proof of a viral cause for a major proportion of BC would lead to special chemotherapeutic and immunologic treatments in the immediate future. We have evidence that combined chemotherapy and immunotherapy is highly effective in test tube experiments. Proof of a viral cause would also justify an intensive effort to develop a vaccine with an aim of preventing 40% of American women's BC.

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PI Name: Ian Tannock, MD, PhD

Institution: University Health Network, Ontario Cancer Institute

Mechanism: Investigator Initiated Research

Application Title: Pantoprazole as a modifier of drug resistance: effects on intra-tumoral distribution and efficacy of doxorubicin for treatment of metastatic breast cancer

Abstract

The study builds on results from Dr Tannock's laboratory that show that the anticancer drug doxorubicin (Adriamycin) remains close to blood vessels in solid cancers (including human breast cancers grown in mice) and does not reach many of the cancer cells. In contrast doxorubicin achieves a uniform distribution in most normal tissues, thereby putting the cancers at a disadvantage, because it may kill more normal cells than cancer cells. Doxorubicin tends to concentrate in acidic particles within cancer cells, and this limits the amount of drug that is available to enter the cell nucleus, and also the amount of drug that can diffuse further from blood vessels. We have shown that the well-tolerated anti-ulcer drug pantoprazole changes the distribution of doxorubicin in cells by decreasing its concentration in acidic compartments, thereby allowing more of it to interact with DNA in the cell nucleus and kill cancer cells; also more drug is available to diffuse further and reach more of the cancer cells. Pantoprazole was found to increase the effects of doxorubicin to kill cancer cells in tissue culture, and the combination of doxorubicin and pantoprazole caused greater effects to delay growth of human tumors grown in mice than doxorubicin alone, without apparent increase in side-effects. Based on these results we propose to further investigate the combination of doxorubicin and pantoprazole as treatment of human breast cancers growing in mice, and to conduct clinical trials of this drug combination.

Our hypotheses are:

1. Administration of pantoprazole will improve the distribution and effectiveness of doxorubicin in other human breast cancers growing in mice.
2. High doses of pantoprazole will be well tolerated in combination with standard doses of doxorubicin in patients with solid tumors, including women with advanced breast cancer who have received limited prior treatment with drugs like doxorubicin.
3. Doxorubicin and pantoprazole will show promising activity in women with metastatic breast cancer.
4. Pantoprazole will improve the distribution of doxorubicin in tumors of women with breast cancer so that it reaches a greater number of cancer cells.

We plan to evaluate the toxicity of doxorubicin and pantoprazole in mice by giving increasing doses of pantoprazole just prior to doxorubicin, and will study the effects to cause tumor shrinkage and delay in growth after treatment with doxorubicin alone, pantoprazole alone and the combination. We will investigate the optimal time interval between administration of pantoprazole and doxorubicin, and the effects of up to three treatments given at weekly intervals. We will use microscopic examination to study the distribution of doxorubicin in relation to blood vessels of tumors for different human breast cancers growing in mice, with and without pantoprazole. We will also investigate the influence of pantoprazole on autophagy, a cellular process that depends on acidic compartments in cancer cells, and helps cancer cells lacking oxygen and other nutrients to survive and grow.

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In an initial clinical trial, consenting patients with solid tumors, including women with metastatic breast cancer who have received previously limited amounts of drugs like doxorubicin, will receive at 3-week intervals a standard dose of doxorubicin given 30-60 minutes after pantoprazole. The dose of pantoprazole will be gradually increased in successive groups of 3 patients, and they will be evaluated for side-effects and for shrinkage of their tumors. We will also evaluate the time course of pantoprazole and doxorubicin in the blood, to see if one drug influences the other, by taking blood samples at just two times after injection of the drugs.

Once we have found the highest dose of pantoprazole that is well-tolerated, we will perform another clinical trial where women with breast cancer will all receive the same doses of doxorubicin and pantoprazole (using the maximum dose of pantoprazole that was well tolerated in the first trial) to assess the effects against their tumors. Some women with breast cancer will also be asked to have a biopsy of their tumors after treatment (if this can be done safely and with minimal discomfort) to evaluate the effects of pantoprazole on the distribution of doxorubicin in their tumors. If the results of this study appear better than standard treatment with doxorubicin alone, we will ask the company that makes pantoprazole to support a larger trial comparing doxorubicin alone and doxorubicin with pantoprazole. Our long term goal is to use this and similar strategies to increase the effectiveness of chemotherapy for women with metastatic breast cancer.

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PI Name: Xiangshu Xiao, PhD

Institution: Oregon Health and Sciences University

Mechanism: Investigator Initiated Research

Application Title: Targeting CREB-CBP interaction as anti-breast cancer therapy

Abstract

Breast cancer is the most common malignant disease in Western women, with an estimated 182,460 new cases in American women in 2008 alone. It has been established that breast cancer is a group of complex diseases with different molecular signatures. Critical molecules involved in pathogenesis of breast cancer include estrogen receptor alpha (ER alpha) and human epidermal growth factor receptor 2 (HER2), both of which drive breast cancer cell proliferation. Therefore, current drug therapies for breast cancer include inhibitors of ER alpha (e.g. tamoxifen) and HER2 (e.g. Herceptin, a humanized monoclonal antibody against HER2) to arrest cancer cell proliferation. Although these drugs are effective during initial therapy against a subset of patients, a significant fraction of breast cancer patients develop acquired resistance mechanisms to these drugs. In addition, about ~15% of breast tumors are classified as triple receptor negative breast cancer (TNBC), which by definition do not express ER, HER2 and PR (progesterone receptor). These TNBC patients do not respond to either tamoxifen or Herceptin at any time or display de novo resistance. When resistance (either de novo or acquired) arises, the patients are left with rather limited choices for nonselective cytotoxic agents, which kill both cancer cells and normal cells. Therefore, there is a pressing need for anti-breast cancer agents with novel mechanisms of action to target a broad range of breast cancers and overcome resistance seen with current therapeutics. Despite the molecular complexity of breast cancers, they all share two critical characteristics: enhanced proliferation and reduced cell death or apoptosis. Breast cancer cells have many signaling axes or pathways to regulate both proliferation and apoptosis. Therefore, targeting a single axis or pathway is destined to be of limited value in attacking breast cancer because alternative pathways will compensate the one to be targeted. To develop novel agents for better managing breast cancer, we propose to target a converging point of many different signaling pathways with small molecules so that inhibition of this target will simultaneously shut down different signaling pathways to effectively inhibit breast cancer cell growth. A protein called CREB, which is a shortened version of "cyclic AMP response element binding protein," is such a converging point. It is a transcription factor activated by phosphorylation (i.e. putting a phosphate onto a specific amino acid residue). Many different growth-promoting kinases including Akt, MAPK, pp90RSK and PKA could turn on CREB by phosphorylation. Aberrant activation of these kinases is often associated with breast cancer cell resistance to current targeted therapeutics including tamoxifen and Herceptin. Studies in breast cancer samples showed that CREB is overexpressed compared to normal mammary tissues. And the level of CREB expression correlated positively with disease progression and negatively with disease-free survival time. However, no small molecule inhibitors of CREB have been developed to evaluate their potential as anti-breast cancer agents. We recently discovered a small molecule inhibitor called KKI06, which inhibits the interaction between CREB and CBP (abbreviated for CREB-binding protein) and CREB-mediated gene transcription. We have also demonstrated that this compound displays selective toxicity against breast cancer cells without toxic effect against normal cells.

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Therefore, we propose to further characterize this exciting small molecule as potential anti-breast cancer agents by design, synthesis and evaluation of more potent and more water soluble derivatives of KKI06. Success of this proposed research will create a new paradigm for breast cancer management and likely decrease breast cancer mortality.

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PI Name: Michael Lisanti, MD, PhD

Institution: Thomas Jefferson University

Mechanism: Investigator Initiated Research

Application Title: Mining the breast cancer “stromal proteome”: using targeted proteomics to identify novel stromal breast cancer biomarkers

Abstract

Breast cancer is a major cause of death in the United States and the Western World; advanced medical technologies and therapeutic strategies are necessary for the successful detection, diagnosis, and treatment of breast cancer. Here, we show that loss of stromal caveolin-1 is a novel breast cancer biomarker that predicts early disease recurrence, metastasis, survival, and tamoxifen-resistance; we will now explore the association of a loss of Cav-1 in the breast cancer tumor stroma with several other novel human stromal biomarkers that we have identified via unbiased proteomic analysis of Cav-1 (-/-) stromal cells. Interestingly, these novel 15 biomarkers include, 5 myo-fibroblasts markers, one oncogene, one tumor suppressor, and eight glycolytic and metabolic enzymes. Virtually identical studies will be carried out with human DCIS samples; 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.

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PI Name: Richard Kremer, MD, PhD

Institution: McGill University, Royal Victoria Hospital

Mechanism: Investigator Initiated Research

Application Title: Co-targeting parathyroid hormone-related protein signaling and osteoclast metabolism to counter breast cancer metastasis to bone

Abstract

Bone metastases are catastrophic consequences of breast cancer progression that greatly affect patients' quality of life and survival. Skeletal metastases are present in close to 70% of patients who die from breast cancer, and the skeletal complications before death cause considerable suffering due to events such as hypercalcemia, bone deformities, pathological fractures and intractable pain. Breast cancer has a special predilection for spreading to bone because its tumor cells possess a unique capacity to invade the otherwise hostile environment of the skeleton. The cancer tumor cells escape the primary site in the breast and migrate through the bloodstream to the surface of the bones. There, they start local sites of bone destruction by stimulating bone-destroying cells (osteoclasts) which create niches on the bone surface where breast tumor cells multiply and start the skeletal invasion. One of the biochemical factors which gives breast cancer cells the special ability to activate osteoclasts and create these zones of bone destruction is called parathyroid hormone-related protein (PTHrP).

PTHrP is a protein expressed in almost all normal fetal and adult tissues, and plays an important role in growth and developmental processes. But when its levels become dysregulated, PTHrP is associated with oncologic pathologies. In fact, high levels of circulating PTHrP in the blood have been shown to correlate with the more advanced stages of cancer. PTHrP is present at abnormally high levels in more than 90% of skeletal tumors derived from breast cancer and it has been demonstrated that PTHrP is crucial to the local bone breakdown essential to tumor progression. PTHrP is not only involved in the invasion of skeleton through bone destruction, but we suspect it is involved in helping the early stages of migration from the primary site (breast) to the skeleton.

The current treatment against bone metastases is through the use of bisphosphonate drugs such as pamidronate or clodronate. Compounds from this group specifically prevent the bone-destructive action of osteoclast cells. These drugs have proven helpful in reducing bone loss caused by tumors, and in increasing the delay before appearance of new skeletal lesions and fractures. Unfortunately, the number of patients experiencing skeletal complications is only reduced by 20% through pamidronate treatment, which exemplifies the dramatic need for improving the efficacy of current therapeutic approaches.

The present bisphosphonate therapy may lack efficacy because it aims at reducing the bone erosion (osteolysis) caused by osteoclasts, but cannot counter many other crucial aspects of skeletal invasion such as breast tumor cell proliferation and migration mechanisms. The fact that PTHrP is an important control molecule for these mechanisms suggests that its action could be targeted in a novel approach to prevent bone metastases. We therefore hypothesize that a therapeutic approach which would combine the anti-erosion properties of bisphosphonates with a PTHrP neutralization strategy would present greater overall efficacy than the bisphosphonate treatment used as a single agent.

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An efficient method of neutralizing a dysregulated biochemical molecule is through the use of a specific antibody. (A good example is the antibody Herceptin, which targets the overactive control molecule Her2/neu in some cancer cells). We have prepared specific antibodies that efficiently target and bind PTHrP, and greatly reduce cancer progression in mouse model. We propose to test our antibodies by themselves as single agents, and in a combination therapy approach with bisphosphonates in the goal of achieving a better strategy to counter breast cancer metastasis to bone.

The uniqueness of this project resides in (a) the demonstration that PTHrP is involved not only in the stimulation of cancer in the bone environment but in the migration of tumor cells from the breast primary site to the bone, and (b) the use of an anti-PTHrP antibody to target bone metastases and improve the efficacy of the current bisphosphonate treatment by addressing more aspects of skeletal invasion by breast cancer cells.

Importance of outcome: Most patients with cancer will die not because of the tumor in the primary site, but because the cancer has spread to other sites in the body. An estimated 350,000 people die from bone metastases each year in the United States, and a large proportion of them suffer from breast cancer-derived osteolytic metastases. PTHrP action is a crucial mediator in breast cancer cell invasion of bone, and our strategy for targeting this yet-unaddressed biochemical aspect is expected to improve the efficacy of the current bisphosphonate treatment. A combined therapeutic approach resulting in a substantial reduction of bone invasion events by breast tumor cells would represent a significant improvement in quality of life for patients and could result in an appreciable increase in their life expectancy.

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PI Name: Wilson Miskimins, PhD

Institution: Sanford Research/USD

Mechanism: Investigator Initiated Research

Application Title: Targeting metabolic processes in breast cancer cells

Abstract

A nearly universal property of cancer cells is that they have an altered metabolism that helps them to grow, survive under conditions of stress, spread, and evade the immune system. It is this altered metabolism that makes it possible to image breast tumors by PET scanning and it may also make the tumors vulnerable to certain drugs. A large number of drugs have been developed and approved for treating other human diseases that are related to metabolism. One such drug is metformin, which is used to treat type II diabetics. There is recent research indicating that metformin may also be beneficial for breast cancer patients. In our lab we have found that metformin kills most cultured breast cancer cells but not normal cells. We have begun to study the mechanism by which metformin kills tumor cells. Our results support the conclusion that metformin promotes cell death by inhibiting electron transport in the mitochondria. This leads to an increase in reactive oxygen species, damage to mitochondria, and initiation of cell death pathways. It is our hypothesis that normal cells, and a small number of resistant breast cancer cell lines, evade the cytotoxic effects of the metformin because they are able to effectively detoxify reactive oxygen species and efficiently eliminate damaged mitochondria through autophagy. The first aim of the proposal is to test this hypothesis using multiple biochemical, cellular, and molecular approaches, including overexpression and knockdown of specific gene products, confocal imaging, flow cytometry, cell fractionation, and various antibody-based assays. The importance of this aim is that it will provide molecular details of metformin-induced cell death pathways that have the possibility of being manipulated for therapeutic effects. The findings will further substantiate the possibility of using metformin for breast cancer patients. Our work has also provided a rationale for how metformin could be combined with other drugs to improve its performance. In this regard, it is our hypothesis that drugs or compounds that enhance the flux of metabolites through mitochondrial pathways will act synergistically with metformin to promote cell death in breast cancer cells. Using cell culture models we will test compounds and drugs that are expected to promote mitochondrial oxidation of pyruvate or fatty acids. Some of these drugs, like metformin, are already used to treat human metabolic disorders such as diabetes, obesity, metabolic syndrome, or hyperlipidemia. Effects on cell viability and the mechanism of action of these compounds in the presence of metformin will be examined. The final aim is to test metformin, and drugs that show synergism with metformin, in pre-clinical mouse model systems for breast cancer. The effects of these compounds on tumor growth and survival of the animals will be monitored. The expectation is that metformin will slow tumor growth and extend survival time and that this will be enhanced by other metabolism-targeted drugs. We expect that the information obtained by this work will be of value in determining if metformin, together with other drugs that affect metabolic processes, can be of use in breast cancer therapy. This is appealing because metformin, and several of the other compounds that will be tested, are safe and have already been approved for treating other human diseases. This is important because it means the period of time necessary for approval for treating breast cancer patients could be

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significantly shortened. Another important outcome of the proposed experiments is that they will add to our basic understanding of tumor cell metabolism and how it can be manipulated to improve the survival of breast cancer patients.

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PI Name: Daniel Kestler, PhD

Institution: University of Tennessee Health Science Center

Mechanism: Investigator Initiated Research

Application Title: Development of ODAM as a biomarker in breast cancer

Abstract

The ODAM (Odontogenic Ameloblast Associated Protein) gene which resides on human chromosome 4q13.3 and codes for a protein normally expressed during late dental development and in certain glandular tissues is also expressed in breast tumor tissue. ODAM was first detected as misfolded (amyloid) protein fragment in a rare dental tumor (CEOT). We have since developed antibody and DNA reagents to human ODAM and using these tools we have observed its expression in dental tissues and associated tumors, in epithelia tissues from certain glands including mammary, as well as trachea and bronchial tissues. Additionally, ODAM expression was observed in human tumors employing immunohistochemistry (IHC), including those of breast, and lung, while others have reported ODAM in gastric tumors. Notably, breast tumors contain a range of diverse protein expression, resulting in multiple tumor types associated with the malignancy. Thus, there is a continuing need to validate unique proteins expressed in breast cancer which help identify this diversity and through these newly associated molecules provide additional resources for the detection evaluation and treatment of breast cancer.

In addition to detecting ODAM expression in breast tumor tissue we have also observed anti-ODAM antibody reactivity in patient serum. This finding supports a possible clinically relevant immunological response to ODAM tumor expression by the patient. To date, we have evaluated 240+ retrospective breast tumor samples for which our findings link ODAM tumor expression with disease stage and increased patient survival.

The scientific rationale for our proposed work are not simply theory based but are founded upon our findings of ODAM tumor expression and anti-ODAM antibody reactivity in breast cancer patients. Thus, we plan to verify the prognostic and other clinically relevant importance of ODAM expression in tumor tissue as well as to determine the relationship of patient anti-ODAM antibody reactivity to clinical data including their disease stage, survival, recurrence, and cancer status. This will allow us to ascertain potential diagnostic, prognostic, predictive, or possible therapeutic roles for either ODAM tumor expression or patient anti-ODAM humoral response we observe in breast cancer.

We hypothesize that ODAM is a novel biomarker for breast cancer that shows progressive (nuclear) staining in tumor tissue, with loss of cell border expression in stages I-IV, whereby the resultant tumor expression associates with disease staging and survival. We further hypothesize that ODAM functions as a molecule in breast cancer that elicits specific antibodies in a significant proportion of patients which may have predictive, prognostic, or therapeutic relevance. We project that both ODAM expression in breast tumor tissue and resultant patient antibodies will associate with disease stage and show measurable effects on survival.

The research aims of our proposal are to verify the relationship of ODAM expression to disease stage, survival and other available patient data as we have initially reported for tumor biopsies of stage 0-IV patients. Secondly, we plan to verify our observation of anti-ODAM antibody reactivity in patient serum and to further investigate its relationship to

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patient tumor disease stage, survival, and other clinical data. This will allow us to evaluate the possible diagnostic and prognostic importance of this humoral response in patients. Further, we will determine any statistical associations between ODAM expression in breast tumor biopsies and anti-ODAM antibody titers with regard to relevant patient clinical and pathological data. These analyses will aid in determining further clinical applications of ODAM reagents. Finally, we intend to characterize high titer patient anti-ODAM antibodies with respect to their Ig type, ODAM-antigen binding, reactivity with ODAM+ breast tumor tissue, and the potential tumor killing abilities with ODAM+ tumor cells. This will allow us to define the range of Ig types and delineate potent immune regions on the ODAM molecule which elicit an antibody response in breast cancer patients. Further, this will also allow us to ascertain if patient anti-ODAM antibodies function as tumor killing agents which would support a future use of ODAM derived reagents in a therapeutic role as has been proposed for other breast cancer patient derived tumor immune reagents.

Although progress has been made in early detection and treatment of breast cancer, up to 40 percent of surgically treated patients for early stage disease will experience recurrence within 5 years due to hidden metastasis. Our proposal seeks to develop an understanding of ODAM expression in invasive and metastatic breast tumors and patients' ODAM-specific humoral response. This study will provide the basis for the possible direct use of ODAM based reagents in the assessment and treatment of breast cancer within the next decade.

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PI Name: Khandan Keyomarsi, PhD

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

Mechanism: Investigator Initiated Research

Application Title: CDK2 is a novel target for triple negative breast cancer

Abstract

Triple negative breast cancers (ER, PR, and Her2 negative) account for 15% of women affected by the disease. These aggressive tumors correlate to high recurrence rates, shorter survival times. Despite standard chemotherapy there are no additional measures triple negative women can take to protect themselves from a future recurrence.

We have made the significant discovery that 70% of all triple negative patients overexpress alternate forms of cyclin E, referred to as LMW cyclin E. The LMW-E is tumor specific and predominantly found in triple negative breast cancer using 340 breast cancer patients. Additionally, The LMW-E forms are oncogenic and their oncogenicity requires the action of CDK2.

These findings could have a significant impact on the management of breast cancer patients; LMW-E could be used both to identify patients at high risk for recurrence and to select appropriate selective agents for those high-risk patients. On the basis of LMW-E findings, patients not at high risk for recurrence could be spared the unnecessary toxicity of systemic therapy, and patients at high risk for recurrence could receive therapies targeted specifically at cyclin E LMW-E.

We hypothesize that LMW-E expression leads to generation of triple negative breast cancer and that targeting the LMW-E will provide novel therapeutic strategies for triple negative breast cancer

To address this hypothesis we will pursue the following two aims:

Aim 1: Identify the key genes and proteins that drive the aggressive phenotype of LMW-E induced triple-negative breast tumor.

Aim 2: Use of in vitro and in vivo xenograft and transgenic mouse model systems to design most effective treatment strategies targeting LMW-E in triple negative breast cancer

By comparing differences between triple negative cells expressing LMW cyclin E to other breast cancer cells using biological arrays we can determine which genes or proteins are turned on or off. Uncovering unique pathways in this breast cancer subtype will lead to the first additional targeted therapy against the LMW cyclin E available to triple negative breast cancer survivors.

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PI Name: Khandan Keyomarsi, PhD

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

Mechanism: Investigator Initiated Research

Application Title: LMW-E, a novel indicator of Letrozole resistance in breast cancer

Abstract

Endocrine therapy is the treatment of choice for patients with hormone-sensitive breast cancer; however, over time many patients become resistant as tumors develop the ability to escape the antiproliferative effects of endocrine therapy. There is currently no alternative treatment strategy that could be offered to these patients who will most likely have a poor outcome. Understanding the various mechanisms responsible for the development of resistance to estrogen deprivation will identify new therapeutic strategies to enhance the efficacy of breast cancer treatment. We have shown that a mechanism involved in the expression of the low molecular weight (LMW) forms of cyclin E and that the complexes that LMW cyclin E form with their catalytic subunits (CDK1 or CDK2) are resistant to inhibition by p21 and p27. We have also shown that the sensitivity of breast cancers to aromatase inhibitors is altered by deregulation of cyclin E. Based on these results we hypothesize that tumor cells that overexpress the LMW cyclin E may be more resistant to aromatase inhibitor therapy than those cells that do not express the LMW cyclin E. The overall objective of this proposal is to identify an accurate predictive marker for endocrine therapy in breast cancer patients, using cyclin E deregulation as the functional target. We will do this through a clinical trial and in vitro using cell lines. We will test our central hypothesis through the following specific aims:

- 1) Establish cyclin E as predictor of clinical response to neoadjuvant aromatase inhibitors in post-menopausal women with clinical stage II-III ER positive breast cancer.
- 2) Determine the mechanisms of reduced sensitivity to aromatase therapy in cyclin E over-expressing breast cancer cells in vitro and in vivo.

The information gained through the proposed studies may have tremendous clinical relevance for post-menopausal women with early stage and advanced breast cancer. We already know that cyclin E overexpression correlates with poor outcome; if cyclin E overexpression also predicts response to endocrine therapy, then clinicians can more appropriately tailor systemic treatment in combination with biological modifiers (i.e. CDK1/CDK2 inhibitors) to those at greater risk of metastasis and death. The information gained through this proposal may therefore contribute to a more complete understanding of the role of cyclin E in modulating the efficacy of endocrine treatment in cancer patients and selective targeting of deregulated cyclin E.

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PI Name: Suzanne Fuqua, PhD

Institution: Baylor College of Medicine

Mechanism: Investigator Initiated Research

Application Title: Role of Dicer and BCRP in hormone resistance

Abstract

Unfortunately many breast cancer patients will develop therapy-resistant disease, and subsequently develop lethal distant metastases. This remains an devastating clinical problem which needs new strategies to overcome. With this goal in mind, we performed a pilot microarray experiment to identify new mechanisms of hormone resistance. We discovered that a gene called Dicer was expressed at higher levels in metastatic, hormone-resistant ER-positive human breast tumors, and we will determine if it is a new biomarker of resistance in this proposal. We have preliminary data already showing that breast cancer cells become resistant to the antiestrogen tamoxifen (Tam) when we overexpressed Dicer. We observed a concomitant increase in expression of the breast cancer resistance protein (BCRP) when Dicer was overexpressed. We therefore examined whether Tam resistance may involve increased levels of BCRP. Indeed, we found treatment with BCRP inhibitors restored Tam sensitivity. Furthermore, we discovered that if we grew cells (both control and Dicer overexpressing cells) in mammosphere assays (a method to enhance for stem cell-like cells), we enhanced tumor initiation and metastasis, with as few as 5 mammosphere-selected cells initiating tumor formation and distant metastases in mice. These cumulative results suggest that Tam treatment of Dicer-overexpressing breast cancer cells enriched for cells with enhanced BCRP function, and growth in the mammosphere assay could provide a new method allowing for the development of ER-positive metastases. We hypothesize that BCRP, via Dicer up-regulation, may be a novel clinical target to reverse resistance.

We therefore propose studies to determine if high levels of Dicer and BCRP confer resistance to hormonal therapies, to examine the mechanisms associated with metastasis and resistance, and to determine whether Dicer and BCRP expression is prognostic of distant relapse and poor outcome in breast cancer patients, so that clinical therapeutic interventions thwarting BCRP's effects can be developed. Our proposed aims and how we will test them are:

(1) To determine how Dicer overexpression affects estrogen receptor (ER) function and hormone response. Dicer will be overexpressed in ER-positive breast cancer cell lines, and examined for effects on Tam response (in vitro and when grown as tumor xenografts in mice), and effects on known growth pathways through the ER will be examined. To test for effects on response to aromatase inhibitors, cells stably expressing aromatase have been generated, and will be stably co-transfected with the Dicer expression vector. We will investigate the involvement of the potential intermediate, Notch, and we will also identify Dicer targets using mRNA expression microarrays and microRNA arrays.

(2) To determine how Dicer affects BCRP and metastatic behavior. We will first determine if BCRP is required to modulate intracellular Tam levels in tumors from Dicer-overexpressing cells, and we will use specific BCRP inhibitors and knockdown of BCRP levels to validate that

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BCRP is the relevant clinical target. We will study metastasis in our models, and as a control use ER-negative cells with Dicer introduced to study how BCRP is upregulated with Dicer overexpression. It is possible that the effects of Dicer on BCRP are due to altered miRNA expression or protein stability; these mechanisms will be examined as well.

(3) To determine if Dicer or BCRP expression are prognostic markers of distant relapse, and/or predictive markers of hormone response, using retrospective analyses in breast cancer patients. We will conduct a retrospective study of tumors from women treated with Tam, with long-term clinical follow-up. We will use immunohistochemistry in this cohort to determine if Dicer or BCRP levels are associated with resistance. We will establish human tumor xenografts in mice to establish a long-term culture system from patients resistant to Tam or aromatase inhibitors, and use these new models to examine the role of Dicer and BCRP in resistance.

These studies could lead to completely new approaches to treat therapy-resistant cancers. By blocking BCRP with specific inhibitors, we will enable women to continue hormonal therapies to successfully fight and eradicate distant metastases. It is also possible that BCRP inhibition combined with hormonal therapy could be useful as an adjuvant therapy as well, to prevent the emergence of lethal resistant, metastatic disease. These are tangible and realistic outcomes from successful completion of this proposal. The project is innovative and is aimed at a novel target we discovered in patient samples from women with metastatic cancer. Thus, we propose a highly translational project and have assembled a panel of scientific experts to tackle this important clinical problem.

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PI Name: Hannah Linden, MD

Institution: University of Washington School of Medicine

Mechanism: Investigator Initiated Research

Application Title: Pharmacodynamic and tissue measures of early breast cancer endocrine sensitivity

Abstract

Anti-estrogenic endocrine therapy has improved outcomes for breast cancer. Unfortunately, up to 50% of breast cancer patients do not respond to this therapy, so even after following the recommended path of treatment, patients may experience recurrence or metastasis. Patients who do respond have a low risk of relapse and may benefit from a treatment program of endocrine therapy only, allowing them to avoid chemotherapy and its many side effects. This study evaluates a rapid test that determines who benefits from endocrine therapy and who should proceed to a different therapeutic approach. The test could be administered within days or weeks after the start of treatment, and unlike current biopsy-based tests, it is non-invasive. It uses positron emission tomography (PET), a common scanning technique already available in many major hospitals and treatment centers. PET imaging allows multiple tumor sites to be visualized in one scan, and since it simultaneously measures metabolic activity, it provides more information than just tumor size. An advantage of this study is that enrolled patients will receive their own test results as they are generated, providing them with additional information that can be used to design the most effective treatment program for their situation.

Preliminary work by scientists at the Seattle Cancer Care Alliance and Fred Hutchinson Cancer Research Center shows that PET imaging can measure the response of a tumor to endocrine therapy by measuring its uptake of a molecule needed for DNA replication, a process required for tumor growth. The more active the tumor, the more the cells take up the ¹⁸F-fluorothymidine (FLT) molecule, a sign that they are dividing rapidly. A tumor that responds to endocrine therapy will slow down its rate of growth and take up less FLT after treatment. The researchers in this study hypothesize that the response of a particular tumor to endocrine therapy can be determined less than 2 weeks after the start of endocrine therapy, by using PET to follow uptake of FLT by tumors. The hypothesis will be tested with a clinical study of patients with early stage breast cancer whose recommended treatment will include endocrine therapy with an aromatase inhibitor (AI). These patients will be treated with an AI during the window of time between diagnosis and their definitive surgery. The FLT PET imaging step can be incorporated without interrupting the patient's treatment plan, and the results will be compared to the currently used standard tests for tumor proliferation.

Following a standard core biopsy for diagnosis, patients will undergo an FLT PET scan, to collect baseline information; a standard proliferation test (Ki-67) will be run on the core biopsy. They will then begin AI therapy as directed by their physician, and undergo a second FLT PET scan to test the effectiveness of the therapy against their tumor, or tumors. After surgery, the removed tumor tissue will be tested by the currently used proliferation test. Based on promising, preliminary results, the researchers predict the FLT PET method will be an improvement over the standard method of testing the response to endocrine therapy, for

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several reasons. First, FLT PET is non-invasive and PET scans are non-confining. Second, a single FLT PET image can provide a direct measurement of tumor activity at multiple tumor sites, and it can be obtained within the 2-week window that typically occurs between diagnosis and surgery. The researchers in this study see this as a "window of opportunity" because a PET scan at this time would not disrupt the regularly scheduled treatment, but would provide pre-surgery information that could result in more efficient decisions about post-surgery chemotherapy options.

If the preliminary results are correct, and PET with FLT is as effective at measuring response to endocrine therapy as predicted, this test could lead to a reduction in breast cancer-associated mortality by reducing the number of patients who are subjected to treatment that is not effective against their particular cancer. Those patients who are responsive may be able to avoid an unnecessary chemotherapy. The ability to make a quick decision about after a short course of therapy will lead to a more personalized approach to treatment. In addition to immediately affecting current breast cancer patients, this study will advance our future evaluation of breast cancer therapy and its effectiveness by taking advantage of new techniques to measure molecular changes in small amounts of tumor tissue. Each pre- and post-surgery tumor sample will be examined for differences in gene expression after endocrine therapy. This will measure changes in proteins and other cell components affected by endocrine therapy, and may help develop future treatments and tests by discovering genes that respond to endocrine therapy. These genes and the proteins they encode are possible targets for future chemotherapies that are more specific and cause fewer side effects.

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PI Name: Mats Lambe, MD, PhD

Institution: Karolinska Institute

Mechanism: Investigator Initiated Research

Application Title: Pregnancy-associated breast cancer; timing and mode of diagnosis, tumour characteristics, prognosis and risk factors

Abstract

BACKGROUND

Breast cancer during pregnancy represents a clinical dilemma and can have a devastating effect for both the mother and the unborn child. Because women in many countries postpone childbearing to older age, the incidence of breast cancer coinciding with pregnancy will increase. In the United States, the number of first births per 1000 women 35-39 years of age increased by 36 percent between 1991 and 2001, and the rate among women 40 to 44 years leaped 70 percent. In the age group 25-29 years where childbearing is most frequent, one in four breast cancer coincides with pregnancy or lactation.

Pregnancy-associated breast cancer (PABC) commonly refers to when the diagnosis of a breast malignancy is made during pregnancy or within one or two years following delivery. The majority of studies to date on PABC have been based on small case series or selected materials. Following a 2003 National Cancer Institute Workshop on reproductive events and breast cancer, PABC was identified as an important area where there is a gap in the clinical understanding that negatively affects optimal management and outcomes.

The goal of the proposed project is to reduce breast cancer mortality in young women by improving the understanding of pregnancy-associated breast cancer. This will be achieved by addressing outstanding questions of clinical importance, including assessment of mode and timing of diagnosis, tumor characteristics, prognosis and possible indicators of risk.

THE FOLLOWING HYPOTHESES WILL BE TESTED

- 1) The diagnosis of breast cancer during or around pregnancy is delayed
- 2) Pregnancy-associated breast cancers are histologically similar, but have specific prognostic markers and are larger and at a more advanced stage at time at diagnosis, compared to breast cancers diagnosed in women of the same age with no recent birth
- 3) Survival in pregnancy-associated breast cancer is poorer than in women with breast cancer of the same age with no recent pregnancy
- 4) The risk of developing PABC is higher in women with familial breast cancer, and the risk is influenced by markers of elevated levels of estrogen exposure during pregnancy, i.e. characteristics of the index birth or index pregnancy

RESEARCH AIMS AND DESIGN

We have concluded a project in which a study cohort was assembled based on record linkages between several Swedish population-based registers (the Cancer Register, the Multi-Generation Register, the Medical Birth Register, the Cause of Death Register, the Migration Register and Census data). This data was used to identify more than 1100 cases

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of PABC and detailed assessment of incidence trends of PABC in women aged 15-44 between 1963 and 2002. This material forms the basis for the present project proposal. For the purpose of the present project, we will retrieve additional information on 1161 identified cases of PABC by record linkage to Clinical Quality Registers for breast cancer and review of medical records and pathology reports. Based on these data, assessments will be made regarding the clinical presentation of PABC and the timing and mode of diagnosis. Tumor characteristics (histopathology, tumor size and stage) and survival will be compared between PABC cases and women with breast cancer in the same age with no recent birth. In addition, tumor specimen will be retrieved from pathology laboratories for identified PABC and non-PABC cases for analyses of prognostic markers. To examine possible risk factors for PABC, we will perform a nested case-control study, including the role of family history and reproductive factors using data from the Multi-Generation and the Medical Birth Register.

ADVANCEMENT OF THE UNDERSTANDING OF BREAST CANCER AND IMPACT ON MORTALITY

Based on a uniquely large population based dataset, the present study will improve the understanding of pregnancy-associated breast cancer by addressing a range of outstanding questions of relevance for the prevention, detection and treatment of breast cancer in this young patient population. If breast tumors occurring during and shortly after pregnancy have special characteristics, this will improve the understanding of underlying mechanisms, and possibly also affect treatment decisions. An improved general understanding of PABC and an increased awareness among care givers and the public can reduce the mortality in this special group of young breast cancer patients, not only by avoiding undue delays in diagnosis and treatment, but also by providing a basis for the development of evidence-based guidelines for management and tailored treatments. If specific risk factors can be identified, young women at high risk can be identified and screened, which could further reduce mortality. Given adequate dissemination of the results to the general public and the medical profession, impact on mortality could be immediate, and achieved within 10 years. While the target patient population is relatively small, the number of years of life to be saved can be substantial.

THE IMPORTANCE OF THE RESEARCH TO PATIENTS WITH BREAST CANCER

Breast cancer coinciding with childbearing has been denoted the “ultimate challenge” by posing extremely difficult questions both to the caregiver and the patient and her family. The collective results of the present project will help improve management and treatment in young women with breast cancer, thereby reducing suffering and mortality in this special patient group.

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PI Name: Gauri Sabnis, PhD

Institution: University of Maryland School of Medicine

Mechanism: Career Catalyst Research

Application Title: Combination of histone deacetylase inhibitor entinostat and aromatase inhibitor letrozole in ER-negative breast cancer metastasis model

Abstract

Most breast cancers (about three fourth) contain estrogen receptor (ER), a protein inside the cell that receives signal from estrogen to grow. Such tumors that have ER are sensitive to drugs that either bind to the ER and block estrogen from activating it (antiestrogens such as tamoxifen) or block production of estrogen by inhibiting an enzyme, aromatase, which makes it (aromatase inhibitors such as letrozole). Together antiestrogens and aromatase inhibitors are called endocrine or hormonal therapy. Endocrine therapy can be given daily from at least 5 years from the time of diagnosis (adjuvant setting) plus it has few side effects compared to chemotherapy and is well tolerated by patients.

But, about one fourth of the breast cancer patients' tumors do not have ER and can only be treated with chemotherapy, which has many side effects. These (ER negative) breast cancers are also very aggressive and tend to spread to other organs (metastasis) such as lungs, bones and brain. It is widely accepted that mortality following breast cancer is mainly due to metastasis and not the primary tumor.

Hence, newer treatment strategies are required that will not only inhibit the growth of primary tumor but also prevent metastasis formation and growth.

The proposed research is aimed at expanding the application of endocrine therapy to ER-negative breast cancer patients with the addition of histone deacetylase (HDAC) inhibitor. HDAC inhibitors are targeted therapies in development various cancers such as leukemia, lung, breast and prostate. HDAC inhibitors are given in the pill form and have few side effects. These compounds alter functional epigenetic modifications. "Epigenetics" refers to differences in the DNA that are not inheritable. Epigenetic changes include modifications of DNA and/or its associated proteins (such as histone proteins that are attached to the DNA) that have roles in transcription regulation. Chemical modification of these proteins help determine if the DNA is "open" and ready to make a protein (such as ER) or "closed" and can not make a protein. The best examples of transcription modulating epigenetic modifications is DNA methylation and deacetylation of histones.

Several reports have shown that loss of ER expression in ER-negative breast cancer is due to epigenetic modification and this loss can be reversed by HDAC inhibitors. Higher activity of the HDAC enzyme causes the DNA to switch to "closed" position and hence not make the ER protein. This has been seen in ER negative breast cancer tumors. Thus HDAC inhibitors can reduce HDAC activity and turn the DNA in "open" position and make the ER protein. This will result in the tumors that are not responsive to endocrine therapy.

In our laboratory studies, we have shown that HDACi entinostat (MS-275), causes the ER negative breast cancer cells to express ER and aromatase. These cells then become sensitive to aromatase inhibitors (aromatase inhibitors inhibited the growth of the cells that were not responsive before HDAC inhibitor). In the current study, we plan to do a series of laboratory experiments using a mouse tumor model to study the effectiveness of the combination of the HDACi entinostat with aromatase inhibitor letrozole. We also plan to

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study the effectiveness of the combination on the metastasis using a mouse model of lung and bone metastasis. The metastatic growth of cancer cells will be monitored using bioluminescent imaging. The cancer cells will be tagged with firefly luciferase, which produces light that can be photographed with a camera. This way we can monitor the growth of metastasis without having to sacrifice the mice (traditionally mice are sacrificed to measure the number of metastasis present in the mouse's body).

The results of our study will not only help extend the benefits of endocrine therapy to ER negative breast cancer, but also help understand how we could best utilize these novel HDAC inhibitors. We propose that combining of HDACi and endocrine therapy will provide significant benefit in the treatment of ER-negative breast cancer. These proposed investigations will set the stage for future clinical trials with these HDAC inhibitors and endocrine therapy. This research will help improve the quality of life of ER- patients, who would otherwise be limited to toxic chemotherapy.

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PI Name: Karin List, PhD

Institution: Wayne State University

Mechanism: Career Catalyst Research

Application Title: Matriptase-mediated signaling in breast cancer as a target for therapeutic intervention

Abstract

Breast cancer strikes one out of nine women in industrialized Western societies including the United States, and therefore represents a serious health problem. The majority of deaths due to breast cancer are caused by breast cancer metastasis to distant sites such as the lung and bone. Once the cancer has spread beyond the breast, the relative 5-year survival rate drops from 98% to 26%. Clearly, these statistics tell physicians and scientists that improvements need to be made in the treatment of patients with advanced disease. Many breast tumors have elevated levels of proteases, protein-cutting molecules that help the tumor invade tissue, spread to distant sites and establish their own blood supply. Matriptase is a recently discovered enzyme of the protease class that breaks down extracellular protein components, and is located on the surface of the cells that form the ducts of the mammary gland. Matriptase is found at higher levels in most women with breast cancer than in healthy women and a high level of the protease in the breast tumor can predict a more aggressive tumor that is more difficult to treat.

Our hypothesis is that matriptase acts as a so-called signaling protease i.e. it promotes breast cancer progression by cleaving and thereby activating an important growth factor called Hepatocyte Growth Factor (HGF). When active HGF binds to its receptor (c-Met) on the surface of breast cancer cells it elicits a variety of pro-cancerous signals that are involved in cancer cell growth, survival and invasion.

In this proposal we will test our hypothesis using several different model systems including a mouse model of breast cancer and human breast cancer cell lines. We will study the consequences of eliminating or inhibiting matriptase for breast cancer both within the mammary glands of mice and in cell culture. We have developed a new and unique genetic system where we can isolate normal and cancerous mammary cells from mice and induce a deletion of the gene which encodes matriptase (a technique called conditional gene “knock-out”). This way we can compare the ability of cancer cells that have matriptase with cells that lack matriptase to grow and invade in culture and in animals by transplanting the cells into the mammary glands of healthy recipient mice.

Human cultured breast cancer cells will also be used to assess the importance of matriptase for growth and invasion via activation of the HGF/c-MET cell signaling pathway. In these cells we will “knock down” matriptase expression using a molecular tool called small hairpin RNA and by addition of both synthetic and natural inhibitors that inhibits the enzymatic activity of matriptase.

Importantly, we will also determine the localization of matriptase and c-Met in breast tumors from breast cancer patients. This will provide us with important information regarding whether matriptase mediated HGF/c-Met signaling is a general phenomenon or whether it is confined to certain areas of the tumors such as the invasive edges.

By using different model systems and breast cancer cells of diverse origin we will be able to provide a comprehensive characterization of matriptase and its role as a signaling protease

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in breast cancer. This information is pivotal in order to identify matriptase as a key player in breast cancer and thus assess the potential clinical benefits from abrogating its activity.

In the short term, this study will provide us with important new knowledge about the role of matriptase in breast cancer, and we will know whether matriptase is a valid candidate target for new therapeutic drug development. Looking ahead, identification of matriptase as a new target and an alternative approach for inhibition of breast progression may provide an as yet unexplored avenue that could benefit patients suffering from this devastating disease in the future.

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PI Name: John Hawse, PhD

Institution: Mayo Clinic and Foundation, Rochester

Mechanism: Career Catalyst Research

Application Title: The role of ER-beta and endoxifen in the treatment and progression of breast cancer

Abstract

Tamoxifen is one of the most important treatments available for women with estrogen receptor (ER) positive breast carcinomas as it has received approval from the Food and Drug Administration for use in the full spectrum of this disease. Tamoxifen and its metabolites are known to function by binding to ERs, thereby blocking the proliferation inducing effects of estrogen in cancer cells. Although this drug has been used for over 30 years, new information has recently become available regarding the effectiveness of tamoxifen treatment and the way it is metabolized by patients. Results of recent studies from our laboratory, and past clinical studies conducted both here at the Mayo Clinic and at other institutions around the world, indicate that a specific metabolite of tamoxifen, called endoxifen, is the most important determinant of how much benefit women receive from their tamoxifen therapy. More specifically, women who produce low levels of endoxifen see little benefit and have a significantly higher risk of breast cancer recurrence while women producing high levels of this metabolite receive significant benefit and have much better outcomes (2-10).

Nearly all of the studies conducted to date regarding the role of ERs in breast cancer have focused on only one of the two major ER isoforms, ER-alpha (ERa). The second ER isoform, ER-beta (ERb), was discovered in 1996, and was found to be a unique gene with different functions. Shortly thereafter, a number of investigators sought to analyze its role in breast cancer, but unfortunately, made little progress. More recent clinical studies analyzing small patient populations suggest that the presence of this receptor in tumors results in better outcomes following hormone therapy (11-20); however, others suggest either decreased benefit or no benefit from the presence of this receptor (21-23). Since tamoxifen remains the most important therapeutic agent worldwide for breast cancer treatment and since ERb has unique actions compared to ERa, it is critical to further define the role of ERb and endoxifen in the treatment and progression of this disease. The importance of these studies are further magnified by the fact that, based on our recent data demonstrating that endoxifen is the most important tamoxifen metabolite responsible for eliciting the anti-breast cancer effects of this drug, the National Cancer Institute has begun synthesizing endoxifen for eventual use as an alternative treatment for breast cancer patients.

Based on our past studies and our preliminary data, the central hypothesis of this proposal is that the effectiveness of endoxifen as an anti-breast cancer drug is enhanced by the expression of ERb. I plan to test this hypothesis by first determining the ability of endoxifen to block breast cancer cell growth and tumor formation through the actions of ERb. These effects will be compared to cells that only express ERa, or to cells that express both isoforms of the ER. This information is important since there are groups of patients whose tumors express either ERa alone, ERb alone or a combination of the two. In collaboration with my clinical mentors, we will also determine the percentage of breast tumors that actually express ERb, and correlate its expression with the outcomes of patients who have been

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treated with tamoxifen. These studies will be extended to determine if endoxifen concentrations in these patients are also a predictor of tamoxifen effectiveness as is the case for individuals whose tumors are ERa positive. The studies proposed in this application are unique for a number of reasons. First, very few laboratories are currently investigating the role of ERb in breast cancer, and no one is examining the effects of endoxifen on breast cancer cells when acting through this receptor. Secondly, this laboratory was the first to definitively show that ERb functions differently than ERa at the level of gene expression in breast cancer cells (24). Thirdly, our group here at the Mayo Clinic has pioneered the large majority of work involving the mechanisms of action of endoxifen and the importance of this metabolite in patients receiving tamoxifen therapy. Lastly, we have access to thousands of patient tumor samples with known outcomes allowing us to more definitively determine the contribution of ERb as a marker of endocrine responsiveness and as a target of novel therapies. The proposed work is of significant importance to breast cancer patients since the results obtained from these studies will significantly enhance our understanding of the way in which endoxifen functions as an anti-breast cancer agent. This work also has the potential to identify a group of patients whose breast cancer would significantly benefit from tamoxifen or endoxifen therapy based on the expression of ERb. Under the current practices, these individuals would not be treated with highly effective hormone therapies and would instead be targeted for treatments such as chemotherapy which have severe side effects. The completion of these studies is also likely to lead to improved personalized medicine for numerous women world-wide and will pave the way for treating patients with endoxifen, both as a novel adjuvant therapy and as a second line of therapy for those individuals who have failed tamoxifen therapy. Such treatments would ultimately extend or save the lives of thousands of women each year as the identification of patients likely to respond to hormone therapy would significantly increase and the reliance on the body to produce endoxifen following tamoxifen therapy would be avoided.

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PI Name: Kun Ling, PhD

Institution: Mayo Clinic and Foundation, Rochester

Mechanism: Career Catalyst Research

Application Title: Type igamma phosphatidylinositol phosphate kinase: a key molecule promoting breast cancer metastasis

Abstract

Metastasis is the reason that causes death in breast cancer patients. Understanding how metastasis is initiated and progressed is the urgent mission to the development of efficient cancer therapies. Metastatic cancer breast cancer cells are initiated from normal epithelial cells. These cells are bound together by intercellular adhesions that inhibit cell migration and invasion. Malignant cancer cells lost these cell-cell adhesions and get chance to escape from normal breast tissue. Interruption of cell-cell adhesions and development of migratory morphology has been observed highly correlating with carcinogenesis and metastasis in breast cancers. Our goal is to understand the molecules and mechanisms that control the morphogenesis of epithelial cells including two aspects: epithelial polarity and development of migratory phenotype. Towards this aim, we have been focusing on a critical lipid kinase, named PIPKIgamma that generates phosphatidylinositol-4,5-bisphosphate (PI4,5P2). This kinase is necessary for assembly of cell-cell adhesions in normal epithelial cells, however, it is essential for cell motility in cancer cells. More interestingly, PIPKIgamma has been observed to be re-distributed from cell-cell adhesion to cell-matrix adhesion during the epithelial-to-migratory morphologic transition, indicating this kinase may have an important role in the development of metastasis. In this proposal, we will investigate the ways in which PIPKIgamma participates in breast cancer cell migration/metastasis via regulating the generation of PI4,5P2. Ultimately, we hope to translate this knowledge into new strategies for detecting cells where PI4,5P2 signaling is not appropriately regulated, before they have the opportunity to develop into aggressive metastatic tumors. This study will provide valuable information and help us understand the progression of breast cancer metastasis. In this proposal, we will assess the potential of PIPKIgamma to be a biomarker for metastatic breast cancers and breast cancer prognosis. This will be particularly important for the diagnosis and treatment of breast cancers. In addition, we propose that PIPKIgamma could be a promising target of cancer therapy to inhibit or reverse metastatic breast cancers.

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PI Name: William Irvin, MD

Institution: University of North Carolina at Chapel Hill

Mechanism: Career Catalyst Research

Application Title: Validating CYP2D6 genotype-guided tamoxifen therapy for a multiracial U.S. population

Abstract

Breast cancer is one of the largest public health problems facing women in 2009 – this year in the U.S. 182,460 women will be diagnosed and 40,480 women will die from breast cancer. In the U.S., it is the most common cancer in women, the second most common cause of cancer death in women, and the main cause of death in women ages 45-55. Approximately 80% of breast cancers have hormone receptors in the cancer cells, suggesting sensitivity to antiestrogen therapy and eligibility for treatment with the drug tamoxifen. Tamoxifen is one of the most commonly used drugs in the treatment of breast cancer. It is the only approved adjuvant (in addition to surgery) drug for endocrine therapy in pre-menopausal women, and it is also used to treat hormone receptor positive (HR+) metastatic breast cancer; HR + male breast cancer; post-menopausal, HR + breast cancer; treatment of the precancerous condition ductal carcinoma in situ; and to prevent invasive breast cancer in women who are of high risk. Although aromatase inhibitors have proven advantages over tamoxifen in postmenopausal women, in this population recent studies suggest that tamoxifen may be a better drug if the right population is selected. Tamoxifen reduces the risk of breast cancer relapse by 40-50% and the risk of death by 33%; however in spite of this effectiveness, up to 40% of women taking tamoxifen still relapse and die from their disease.

Tamoxifen is a prodrug (needs to be metabolized by the liver to become active) and up to half of those taking it may not receive the full benefit because of genetic differences that affect metabolism to its active form endoxifen. The conversion of tamoxifen to endoxifen is controlled by liver enzymes, including the most important one, cytochrome p450 2D6 (CYP2D6). Many genetic variations of CYP2D6 exist with different abilities to convert tamoxifen to endoxifen. On average, 45% of women have extensive metabolizing CYP2D6 (EM, active CYP2D6), 50% have intermediate CYP2D6 (IM, reduced but not inactive CYP2D6 activity), and 5% have poor metabolizing CYP2D6 (PM, no CYP2D6 activity). Although most studies have been performed in Caucasian populations, the frequency of dysfunctional genes may differ by race, and may be implicated in the worse survival suffered by African-American women with breast cancer. Studies suggest that tamoxifen-treated women with PM genes have shorter relapse-free survival than those who are EM, prompting some to suggest that all women undergo CYP2D6 testing and tamoxifen not be used in those who are PM. Although women who are IM also appear to have worse outcome than EM, there is little guidance about tamoxifen use in those with impaired, but not absent, CYP2D6 activity.

Tamoxifen is approved for use at both 20mg/day and 40mg/day, however by convention it is dosed at 20mg. We hypothesize that endoxifen levels can be manipulated by genotype-guided dosing of tamoxifen and we aim to determine the groups that would benefit the most from such an intervention. We will examine: 1) if doubling the tamoxifen dose will result in

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increased endoxifen levels in IM gene carriers, 2) the proportion and type of impaired activity CYP2D6 enzymes that occur in a racially heterogeneous population, and 3) the association of endoxifen levels and genotype (EM, IM, or PM) with adverse effects (such as hot flashes). In this multisite phase II interventional study, 382 patients already receiving tamoxifen will be genotyped using the FDA-approved AmpliChip and their baseline endoxifen levels determined. IM and PM women will have their tamoxifen dose increased from 20 to 40 mg for 4 months. At that time endoxifen levels will be repeated and compared to baseline. We will also examine the relationship of endoxifen levels and CYP2D6 genotype to symptoms and quality-of-life.

We believe that some women may receive suboptimal doses of Tamoxifen due to genetic metabolizing differences that might be overcome with a simple increase in dose, and that these women are identifiable by genotype. This project uniquely advances our understanding of breast cancer treatment because it is the first to examine the use of genetics to tailor tamoxifen dosing to answer a therapeutic question relevant for hundreds of thousands of women in the U.S. and worldwide. Optimal use of CYP2D6 to determine tamoxifen dosing would have an immediate impact and reduce breast cancer mortality within the next decade by allowing the individualization of endocrine-targeted therapy to maximize clinical benefit (decrease mortality) and minimize toxicity. This would potentially impact not only the 145,000 women diagnosed with endocrine-receptor positive breast cancer each year, but also the majority of the 50,000 women diagnosed with ductal-carcinoma-in-situ and the tens of thousands of women who are candidates for breast cancer prevention with tamoxifen.

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PI Name: Paraic Kenny, PhD

Institution: Albert Einstein College of Medicine at Yeshiva University

Mechanism: Career Catalyst Research

Application Title: Amphiregulin: biomarker evaluation and functional analysis in estrogen receptor positive breast cancer

Abstract

This proposal focuses on the biology of the most common type of breast cancer, which expresses the Estrogen Receptor Alpha and which generally responds to estrogen receptor inhibitors such as tamoxifen and aromatase inhibitors. We have identified a gene, called Amphiregulin, which is found at high levels in many estrogen receptor positive tumors and which responds to estrogen. Increasing estrogen receptor activity switches on expression of the gene and blocking estrogen receptor activity switches off expression of the gene. The Amphiregulin gene makes a soluble protein that breast cancer cells secrete to their environment. This Amphiregulin protein then binds to receptor proteins on the outside of the breast cancer cell which promote cell proliferation. We believe that Amphiregulin is an important estrogen-regulated factor in breast cancer biology and will explore this further in this proposal. Tumors are often quite “leaky” as their blood vessel supply is often poorly developed and disorganized. Because of this, proteins produced by the tumor can often be detected in the blood stream. This can be useful clinically, because measuring these “biomarkers” can provide useful information about the presence of a tumor, its size and its response to therapy. We have begun to test whether Amphiregulin may be a useful serum biomarker for estrogen receptor positive breast disease. In our preliminary data, we have tested 41 cancer-free women almost all of them were negative for serum Amphiregulin, while 3 patients with Estrogen Receptor positive tumors had high levels of Amphiregulin.

We will test two hypotheses in this proposal:

1. That serum Amphiregulin levels are a useful biomarker for the presence and extent of estrogen receptor positive breast cancer and also can be used for monitoring responsiveness to tamoxifen or aromatase inhibitors.
2. That Amphiregulin is a key mediator of the potent growth promoting effects of estrogen on breast cancer cells.

Aim 1. We will enroll 225 patients at Montefiore Medical Center medical center in the Bronx, NY to perform a clinical trial to test our first hypothesis. These women will be from diverse ethnic backgrounds and will represent all subtypes of breast cancer and all stages of the disease. The trial will be minimally invasive as it will only involve occasional blood draws at times when patients are in the hospital for routine therapy. There will be one blood draw for 150 of the patients, while the remaining 75 will experience up to six blood draws over a period of one year. We will measure Amphiregulin levels in the serum samples from these women to determine if Amphiregulin levels are associated with the presence of ER+ breast cancer, whether they change over time and, if they change, whether we can use this to predict response to therapy.

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Aim 2. In parallel, we will examine tumor specimens in detail from 245 breast cancer patients to define the proportion of them that express Amphiregulin and try to determine what other factors may be correlated with Amphiregulin expression (e.g. estrogen receptor status, progesterone receptor status, HER2 status, recurrence risk etc.).

Aim 3. We will also perform a large series of cell biology experiments using advanced 3D culture models of estrogen receptor positive breast cancer cells to explore their requirement for Amphiregulin expression for growth in response to estrogen. This part of the work will define in detail the actual biological function of Amphiregulin.

Aims 2 and 3 will generate interesting new data on the expression, distribution and function of Amphiregulin in breast tumors and in breast cancer cells. This work will provide insight into the basic mechanisms by which these tumors arise and grow and may provide new suggestions for suitable targeted therapies. For example, in other work, we have validated a protease inhibitor strategy for blocking the function of Amphiregulin in breast cancer cells (J Clin Invest 2007; 117 (2) 337-345.).

The most immediate clinical impact is likely to come from the clinical trial in Aim 1. Currently there are no good serum biomarkers to either detect breast cancer or monitor its progression. We predict that Amphiregulin may find use in both of these areas. Also, importantly, once a breast cancer is diagnosed and surgically removed, regular testing for Amphiregulin levels may provide an early warning of an impending tumor recurrence. This may allow patients who are likely to recur to select additional therapy before the recurrence becomes clinically threatening. Finally, although most patients respond to tamoxifen and aromatase inhibitors initially, many patients do not. At present, we have no way of knowing whether a particular patient is likely to respond or not. Because Amphiregulin levels in breast cancer cells are readily modulated by tamoxifen, it may prove possible to evaluate Amphiregulin levels over the first days-weeks of treatment. Patients whose tumors do not appear to show a response to tamoxifen (by experiencing a reduction in circulation Amphiregulin levels) might be encouraged to select additional therapies.

In conclusion, this work addresses the most common breast cancer subtype and offers the potential of developing a better understanding of how estrogen drives these tumor cells and, most importantly, may lead to the availability of a straightforward blood test with many applications in the monitoring of this disease.

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PI Name: Mikala Egeblad, PhD

Institution: Cold Spring Harbor Laboratory

Mechanism: Career Catalyst Research

Application Title: Regulation of the response to cytotoxic chemotherapy by the breast cancer tumor microenvironment

Abstract

Background and rationale:

About 40,000 women die of breast cancer in the United States each year. Many of these women have been unsuccessfully treated with chemotherapy. We know surprisingly little about how cancer cells in tumors react to chemotherapy. Much of our knowledge on how chemotherapy works comes from experiments that do not reflect the complexity of human breast tumors.

The progression of tumors involves a complex interaction between the cancer cells and their surrounding environment, known as the tumor microenvironment. This environment includes structural proteins, growth factors and various supporting, non-cancerous cells. New studies show that the tumor microenvironment plays a role in determining if chemotherapeutic drugs reach the cancer cells. Other factors in the microenvironment (for example growth factors) can also stimulate cancer cells so they become drug resistant. Thus, the tumor microenvironment influences the response to chemotherapy. However, treatment may also alter the microenvironment: chemotherapy has side effects on non-cancerous cells in the body (e.g. in the bone marrow). Up to 80% of breast cancer patients treated with the chemotherapeutic drug doxorubicin develop a severe reduction in the number of neutrophils (a type of immune cells involved in combating infections). Interestingly, the survival from breast cancer is higher for patients that develop a mild reduction in the number of neutrophils than for patients that do not. This may simply mean that the degree of reduction is a surrogate marker for the sensitivity cells, including cancer cells, in individual patients to chemotherapy. Alternatively, chemotherapy might be more effective because immune cells, including neutrophils, are reduced. Neutrophils are part of the family of myeloid derived immune cells. The infiltration of myeloid cells into breast tumors is associated with poor prognosis in breast cancer. Myeloid cells have several known functions that can support the tumor, including stimulation of cancer cell proliferation, metastasis, and angiogenesis.

Hypothesis and expected results:

The influence of the tumor microenvironment on the response to chemotherapy is not well understood. Gaining new knowledge in this field requires novel approaches that allow the study of the dynamic interactions between cancer cells and their environment during treatment. We have developed an imaging technology that permits laser microscopy of breast tumors in live mice while treatment occurs. We fluorescently label cancer cells and their surroundings in different colors using modified genes isolated from jellyfish, so we can observe how and where cancer cells are killed. The tumors that develop in our mouse models resemble two major types of human breast cancer, and importantly, they have a microenvironment that is similar to that of human breast tumors. Animal models are essential for this project, as the imaging technology for technical and ethical reasons cannot

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be used in humans. Preliminary experiments have shown that our technology can detect striking difference in the efficacy of the chemotherapeutic drug doxorubicin in tumors of different sizes and malignancy.

We hypothesize that the tumor microenvironment can regulate the response of the tumor to chemotherapy. The goal of this study is to increase the understanding of how cancer cells in intact tumors react to different types of chemotherapy.

Study design:

We will use live laser microscopy and mouse models of breast cancer to test if and how chemotherapy is regulated by the tumor microenvironment. We will test if breast cancer subtype and size predicts the response to a group of chemotherapeutic drugs. We will test if cancer cells in tumors that have become resistant to chemotherapy become sensitive again if the cells are put in a different microenvironment. We will test two possibly approaches to change the microenvironment to improve the response to chemotherapy. First, we test if we can increase the amount of chemotherapeutic drug that reaches the cancer cells, by targeting the blood vessels in the tumors so more drug get out of them. Next, we will test if the response to chemotherapy changes if we intentionally kill different types of the myeloid immune cells that are present in the tumor before we give chemotherapy.

Importance of research for breast cancer patients:

This project will analyze a clinically significant issue, namely the potential for the tumor microenvironment to regulate resistance to chemotherapy. The overall goal of the project is to determine if we can change the microenvironment of the tumors so the response to chemotherapy becomes more favorable. Positive results in our animal model could quickly result in better treatment for breast cancer because most of the drugs necessary (including the chemotherapeutic drugs and drugs to target the microenvironment) already exist. The project will also result in the development of a new method to test the efficacy of drugs in real-time, which may become useful for future drug development. Together, the experiments we propose could profoundly change our view of how tumors develop resistance to chemotherapy, and thereby influence the choice of treatment for breast cancer and ultimately the survival from the disease.

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PI Name: Megan Troxell, MD, PhD

Institution: Oregon Health and Sciences University

Mechanism: Career Catalyst Research

Application Title: Kinase gene mutations and amplification in breast precursor lesions and progression: potential role in diagnosis and targeted therapy

Abstract

Pathologists diagnose breast cancer by examining tissue samples on slides with a microscope. Cancer can be recognized by the malignant features of the constituent cells, as well as by the relationship of those cells to normal structures (invasion). Malignant cells that have not yet invaded the breast are called carcinoma in situ (CIS). There are also a number of other cell types that do not look like cancer cells, but are also not normal. While pathologists can classify these types of cells based on their appearance under the microscope, we do not yet understand the relationship of these cellular changes to cancer— which lesions are really precursors (precancer)? For instance, we know that the lesion called “atypical ductal hyperplasia” shares some features of CIS, but the lesion called “usual ductal hyperplasia” also has too many cells, but they do not look like cancer.

The development of cancer, including breast cancer, is recognized to be a multi-step process. Scientists have hypothesized that changes in microscopic appearance from normal to hyperplasia to CIS to invasive cancer will have corresponding cellular changes (mutations, mistakes in copying cellular DNA, the material that codes for the structure and function of cells), with more mutations the closer the cell type is to cancer. This is true in many other types of cancer, and many of the mutations are known. However, in the case of breast cancer, we know far less about the individual mutations, although we do know that large pieces of DNA may be gained or lost as cells progress toward breast cancer. There is very little known about how specific mutations map to certain abnormal cell types that are seen with the microscope. Characterizing mutations in breast precursor lesions will help understand the biologic potential of these lesions, and help better distinguish those that warrant treatment from those that do not. Importantly, knowledge of these mutations in breast will allow the development and application of DNA-based diagnostic techniques to breast lesions, and targeted chemopreventative therapy.

We aim to study particular mutations (changes in DNA) in breast cancer and precancer and other abnormal cells in the breast. Based on studies in many other labs, we know certain mutations that increase cell growth, increase cell division, and turn up activity of cells. We know that many of these mutations are found in breast cancer, but we do not know when in carcinogenesis they appear. By studying mutations in a variety of different breast lesions, we aim to identify those breast lesions that have the greatest potential to become cancer, and also to learn which mutations are most important in the development of breast cancer.

We hypothesize that we will find different mutations in abnormal breast cells than we will find in CIS, and invasive carcinoma. We also hypothesize that the type of mutations will suggest new diagnostic and therapeutic strategies for breast precancer and cancer. Also,

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we hypothesize that aggressive invasive breast cancer will have characteristic profiles of mutations, expanding potential for therapy targeted at those mutations.

With an IRB approved protocol, we will study human breast tissue previously removed at surgery. We will identify cases with cancer, CIS, and a defined set of abnormal cell types (known to pathologists as usual ductal hyperplasia, atypical ductal hyperplasia, columnar cell change, flat epithelial atypia, among others), as well as normal cells. We will isolate the cells (using laser-capture microdissection on slides), and we will test the DNA of these cells for a panel of 321 mutations in 30 genes known to be associated with cancer in breast and other organs (using mass spectroscopy technology-Sequenom). We have previously demonstrated that we can apply this technology to screen for this panel of mutations in a very small amount of material in our previous study of breast papillomas. Secondly, we will develop assays to study gene amplification using similar technology amenable to small amounts of DNA.

In the era of screening mammography, more women are having biopsies which show these putative precursor lesions, but we do not have a full understanding of which lesions truly need surgery or drug therapy. Our study should add important data that can be used in diagnosis and clinical management of these lesions. For instance, if a particular 'bad' mutation is characterized in some early lesions but not others, this data can lead to development of DNA assays for clinical diagnosis. In addition, there are now drugs that will turn 'off' cells which have certain mutations, but not affect normal cells (targeted therapy), and these drugs could then be applied to those precursor lesions which warrant treatment. These types of drugs also may come to provide another means of less toxic targeted therapy for otherwise aggressive types of breast cancer, especially breast cancer which has proven resistant to conventional chemotherapy. This project will help better understand the implications of breast precursor lesions, better recognize important precursor lesions, and suggest preventative treatment of those lesions; thus, this project will contribute to a reduction in the incidence of breast cancer. Further, by identifying mutations in cancer that may be amenable to targeted therapy, this project will contribute data leading to reduced mortality from breast cancer.

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PI Name: Rebecca Muraoka-Cook, PhD

Institution: Vanderbilt University School of Medicine

Mechanism: Career Catalyst Research

Application Title: Overcoming resistance to HER2-targeted therapies through inhibition of HER3-induced PI3K activity

Abstract

One of the greatest advances in breast cancer treatment has been the development of molecular-targeted therapies (eg. HER2 inhibitors). Despite these advances, more than 30,000 women in the U.S. die each year from breast cancer. These women often lose their battles with cancer because their malignancy developed resistance to targeted therapies. New therapies are urgently needed that will combat the cause and consequence of resistance to targeted inhibitors.

The HER2/Neu oncogene is overexpressed in approximately a quarter of all patients with breast cancer. In many of these patients, anti-HER2 drugs such as the antibody trastuzumab (Herceptin), or the oral HER2 inhibitor lapatinib are effective, FDA-approved treatments. However, many HER2-positive tumors do not respond to anti-HER2 drugs initially. Furthermore, the majority of responsive tumors eventually become resistant to anti-HER2 treatment. To improve upon the clinical success of HER2 inhibitors, an understanding of the cause of their resistance is required. Discovery of the mechanisms contributing to resistance to anti-HER2 treatments is essential for two important reasons. First, the presence of these alterations should identify patients in whom current anti-HER2 therapies will not work when given alone. Second, the presence of these alterations will inform physicians about other drugs that can be combined with HER2 inhibitors in an effort to increase survival and eventually cure patients with HER2-positive breast cancer.

Studies described in this application are aimed at understanding how HER3, a protein that interacts closely with HER2, contributes to anti-HER2 resistance. Experiments will test the hypothesis that activation of HER3 occurs in response to sustained HER2 inhibition, thus promoting survival of tumor cells, resulting in anti-HER2 resistance. If so, HER3 inhibition in combination with HER2 inhibitors would decrease resistance to HER2 inhibitors, thus lowering the mortality caused by anti-HER2 resistance.

It is plausible that information gained from the proposed experiments would result in the development of successful drug combinations that could alter the standard of care for breast cancer patients within the stated goal of 10 years. Since HER3 inhibitors are in development, the results could promptly impact methods used in patients if our hypothesis is supported.

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PI Name: Elena Pugacheva, PhD

Institution: West Virginia University

Mechanism: Career Catalyst Research

Application Title: The role of NEDD9 protein in proliferation and metastasis of breast cancer

Abstract

The major cause of mortality from breast cancer is spread of the disease to distant sites, called metastasis. Currently, effective treatments against metastasis are not available. One of the reasons is that tumor cells acquire resistance to the cancer drugs during the course of treatment and only handful number of biomarkers for metastatic tumors exists for diagnostic/prognostic purposes. Identification of mechanisms used by tumor cells to avoid the treatment will advance the development of new therapeutic strategies and identify vulnerable steps where successful treatment could be achieved. About 94% of breast cancers have an abnormally high level of a protein called Aurora A (AurA). This protein stimulates cell division and is normally present in the cell for only a short period of time. Recently, several AurA inhibitors have entered clinical trials for cancer treatment. Unfortunately, these inhibitors were found less efficient for solid tumors and the mechanisms of such resistance are unknown. In our previous studies, we have identified several proteins involved in activation of AurA in tumor cells. Among them, a protein called NEDD9 (formerly HEF1) increases both tumor cell migration and proliferation. Recent studies correlate over-expression of NEDD9 specifically with breast cancer metastasis. Our work on mouse models of breast cancer indicates that removal of NEDD9 protein not only limits tumor growth but also eliminates metastasis thus providing us with an efficient means to formulate new treatment strategies for breast cancer patients with metastasis. The objective for this proposal is to determine the mechanism by which high levels of NEDD9 protein substantiates the resistance of breast tumor cells to AurA inhibitors and promotes metastasis. In Aim 1, we will identify molecular mechanisms utilized by NEDD9 to upregulate AurA protein level in tumors. We will address the impact of the NEDD9-AurA interaction on the efficacy of AurA inhibitors used in clinical trials and provide guidance for future AurA based therapies. In Aim 2, we will examine the role of NEDD9 and AurA in tumor invasion and metastasis in tumor cell lines and examine the efficacy of AurA inhibitors in metastasis prevention/treatment. These data will enable us to test if expression of NEDD9 and AurA could be used as prognostic metastases biomarkers in breast carcinoma patients and build a foundation for the application of AurA inhibitory compounds as well as the development of novel small molecule inhibitors of NEDD9.

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PI Name: Michael Hassett, MD

Institution: Dana-Farber Cancer Institute

Mechanism: Career Catalyst In Disparities Research (CC-DR) Grants

Application Title: Quality, disparities, and breast cancer care in medicaid

Abstract

The main tool used by health care organizations, researchers and policy analysts to assess the quality of care provided in the United States is the quality measure – a description of practice performance relative to an established standard. The quality of care offered to breast cancer patients has been studied extensively; numerous circumstances in which patients do not receive effective treatments have been identified. A number of research studies have focused specifically on whether women of different racial groups receive different levels of quality care. They have demonstrated that African-American women are less likely to receive recommended breast cancer treatments compared with white women.

Unfortunately, we know relatively little about the experiences of other under represented racial groups, such as Asians and Hispanics. Do they receive the same quality of care as whites or are their experiences more akin to those of African-Americans? Also, we do not clearly understand the impact of race on quality of care, independent of the influence from other factors, such as poverty. And despite extensive previous research, the pace of improvement in cancer care quality has been slow. This may be partly because there is a disconnect between academic efforts to study these problems and practical efforts to improve quality, so we know more about the types of quality problems that exist than about how to fix them.

In our previous work, we developed a method of using information about what doctors actually do, their practice performance data, to identify areas of clinical care where practice performance is suboptimal and potentially improvable. We believe these are the areas where quality improvement efforts should focus their resources. We applied this approach to medical record information from 10 large U.S. cancer centers, and identified high priority measures for breast cancer care. However, our findings were limited, because only a relatively small fraction of the 9,000 women whose care we analyzed were non-white or poor (and these are the women who are most at risk for experiencing quality problems).

Recently, Dr. Deb Schrag, a researcher and cancer doctor at the Dana-Farber Cancer Institute in Boston, MA, created a new database that contains information on more than 13,000 women with breast cancer, including details of their cancer diagnosis and treatment. Importantly, all these women are insured through Medicaid, so they all have low incomes, and many represent racial/ethnic groups that have not been studied in detail previously. This unique dataset is a potentially invaluable resource for studying the differences in quality of care experienced by women of different races who have breast cancer.

We propose to conduct a research project with the following objectives: 1) to better understand the size of the quality problems experienced by poor women with breast cancer

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who are white, African-American, Hispanic, and Asian; 2) to determine how much of the quality problems experienced by poor women are attributable to their race/ethnicity; and 3) to identify high-priority areas of clinical care where quality improvement efforts are most needed to address the disparities that exist between women of different racial groups. We firmly believe that the results of this research project will enhance national efforts to improve cancer care quality and reduce disparities within the next 3 years.

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PI Name: Cynthia Owusu Owusu, MD

Institution: Case Western Reserve University, School of Medicine

Mechanism: Career Catalyst In Disparities Research (CC-DR) Grants

Application Title: Improving outcomes for older women with breast cancer

Abstract

Recent gains in life expectancy coupled with aging as a risk factor for breast cancer makes breast cancer a disease of older women. Whilst breast cancer mortality rates are declining among women less than 70 years of age, they have either been stable or increasing among the oldest old women with breast cancer. One reason for the existing age-related disparities in breast cancer mortality is the under-treatment of breast cancer among older women in comparison with their younger counterparts. Several barriers have contributed to the under-treatment of older women, including, but not limited to, misconceptions and misinformation about reduced functional reserves and tolerability of cancer treatment by older women. This has served to limit their options and impact their survival..

We hypothesize that age-related disparities will be observed in receipt of recommended standard treatment for breast cancer and that age-related disparities in breast cancer treatment will be partly explained by differences in declines in physical function that occurs during cancer treatment of older women. To examine our hypothesis will require that we measure physical function and ensuing decline that occurs during breast cancer treatment of older women. Given that current instruments used by medical oncologists to measure physical function among cancer patients have been shown by clinical research not be particularly accurate when applied to senior adults, and none of the instruments currently recommended for screening in senior adults with cancer have been validated for this purpose, there remains a critical need for a well validated instrument for functional decline among senior adults with cancer. Furthermore, despite the existence of substantial literature supporting a strong correlation between several biomarkers and reduced functional reserves, use of such biomarkers to evaluate for risk of functional decline and inform the design of interventions for functional decline among senior adults with cancer remain unexplored.

We therefore propose to conduct a study of 200 women 65 years of age and older who have been newly diagnosed with stage I-III breast cancer. As a first step we will develop an instrument for identifying functional decline in our cohort and then use the instrument to examine the mediating role of functional decline on age-related disparities in breast cancer treatment. Specifically we will;1) Determine the ability of the Vulnerable Elders Survey (VES-13) to predict 12-month functional decline or death in women ? 65 years with newly diagnosed stage I-III breast cancer. The VES-13 is a self-administered tool which has been validated in community dwelling older adults to predict 12-month functional decline and mortality. 2) Identify correlative biomarkers of functional decline [inflammatory cytokines, C-reactive Protein (CRP), White Blood Count (WBC), D-dimer, hemoglobin, albumin, and cholesterol] to enhance risk stratification and develop an instrument for functional decline in older breast cancer patients 3) Assess the relation between age, functional decline and receipt of standard breast cancer therapy among older women with breast cancer.

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The is a longitudinal study of 200 women, ? 65 years of age with newly diagnosed stage I-III breast cancer recruited from ambulatory surgical and medical oncology clinics of the University Hospitals Case Medical Center and community satellite facilities, in Cleveland, Ohio. Following enrollment, women will be followed prospectively for 12 months to complete study procedures at four time points as follows: at study entry (baseline), post-surgery, six months and twelve months (study completion). Study procedures include administering the VES-13 and Activities of Daily Living (ADL)/Instrumental Activities of Daily Living (IADL) at all four time points, a Comprehensive Geriatric Assessment at baseline and study completion and biomarker testing at baseline only.

Results from the proposed study will provide new information on the appropriate instrument for measuring functional decline in older women with breast cancer and on the role of functional decline in age-related disparities in breast cancer treatment. Understanding this relation is important for designing strategies for remediation of functional decline. . The ability to correctly identify reduced functional reserves among older women undergoing treatment for breast cancer should be helpful in ensuring that healthy independent older women go on to receive recommended guideline treatment and those with reduced functional reserve receive the necessary remediation that optimizes their oncology care. Over the long-term remediation of functional decline among older women with breast cancer should translate into reductions in treatment differences and improved breast cancer survival.

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PI Name: Kunxin Luo, PhD

Institution: University of California at Berkeley

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Understanding the development of breast cancer: The role of SnoN in the mammary cells function and breast cancer progression

Abstract

Breast cancer is the most frequently diagnosed tumor in women and is the second leading cause of cancer deaths. Invasion to other organs is the major cause of death in these patients. Development of new diagnostic and treatment strategies relies on a thorough understanding of the pathways that regulate mammary gland function and breast cancer development. The growth of normal breast cells is under stringent regulation by various growth factors. One of the key negative regulators of normal breast cell growth is TGF β (Transforming Growth Factor beta).

TGF β plays important roles in normal mammary gland growth and also has a dual role in breast cancer development. In normal breast cells or during early stages of cancer, TGF β suppresses tumor development by inhibition of cell proliferation or promoting cell death. However, the growth-inhibitory activities are often inactivated by mutations during progressive stages of the cancer. When breast cancers reach the later stages, TGF β can promote tumor invasiveness and metastasis through its ability to modulate tumor microenvironment. Therefore, an in-depth understanding of this signaling activity in normal mammary gland development and the complex roles of TGF β signaling in breast cancer progression is crucial to the design of new diagnostic and treatment strategies. The long-term goal of the proposed research is to understand how TGF β signaling plays a role in mammary gland development and breast cancer progression, with a special focus on a critical negative regulator of TGF β signaling called SnoN.

Mammary gland development usually starts during puberty when mammary epithelial cells undergo proliferation. This process continues during pregnancy, and at lactation, functional differentiation of these epithelial cells take place and milk production starts. After weaning, involution phase starts and rapid epithelial cell death remodels mammary gland to the mature virgin state. TGF β is expressed in the virgin glands and plays important roles during pregnancy, lactation and involution. TGF β exerts its functions through its surface receptors and the Smad proteins. Binding of TGF β to its receptors results in the activation of Smad proteins. The activated Smads then accumulate in the nucleus, bind to DNA and activate the expression of TGF β target genes. This Smad signaling pathway is additionally modulated by many cellular proteins. SnoN is a critical negative regulator of TGF β signaling through binding to and inhibiting the Smad proteins. This protein is expressed in normal breast cells at a low level but this expression is significantly elevated in breast cancer cells. Currently it is not clear how the expression of SnoN is upregulated in breast cancer cells and what is the biological consequence of this upregulation in breast cancer development. In addition, the role of SnoN in normal mammary gland development and function remains poorly defined. The goal of this proposal is to understand the function of SnoN and the SnoN/Smad interaction in regulation of mammary gland development and function as well as breast cancer progression. We will combine in vitro mechanistic studies

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in tissue culture models with in vivo studies in animal models. For in vitro studies, we will employ a unique three-dimensional (3D) culture system for mammary epithelial cell differentiation that mimics the basic functional unit of human breast to determine the function and signaling mechanisms of SnoN in normal mammary epithelial cells. We will also confirm what we learn in this in vitro culture system in mouse models to determine the role of SnoN in normal mammary glands at virgin, pregnancy, lactation and involution stages as well as in breast cancer development in vivo. The specific aims are:

Aims 1 and 2: Determining the function of SnoN in proliferation, survival, polarity and differentiation of normal human breast cells using the 3D culture model system. By downregulation of SnoN in normal mammary epithelial cells through a specific siRNA, we will determine the effects of reducing SnoN on the proliferation, survival, polarity, cell-cell adhesion and cellular death of mammary epithelial cells. With this study, we expect to obtain a comprehensive picture of the effects of SnoN expression throughout the entire process of mammary epithelial cell differentiation and how disruption of this regulation leads to the cancerous transformation.

Aim 3: Determining the function of SnoN in mammary gland development and breast cancer in animal models. To complement the biochemical and cell biological studies in cell cultures, we will use SnoN deficient mice, as well as mice overexpressing SnoN to mimic the situation found in human breast cancer. In these experiments we will determine the role of SnoN in proliferation, survival and function of mammary epithelial cells and development of mammary glands. More importantly, we will be able to assess the role of SnoN in promoting breast carcinogenesis in animal model using SnoN overexpressing mice. To determine whether SnoN or the SnoN/Smad interaction accelerates or delays breast cancer, the above SnoN transgenic mice and a well-developed breast cancer mouse model will be crossed and littermates will be monitored for development of mammary tumors and their invasion to other organs, such as lung.

We believe that by conducting this research we will acquire a better understanding of the signaling pathways involved in mammalian tumorigenesis and metastasis of breast cancer. We strongly believe that all this effort will be ultimately beneficial in developing new therapeutic strategies.

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PI Name: Gultekin Gulsen, PhD

Institution: University of California at Irvine

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: A combined mri-dynamic contrast enhanced fluorescence tomography system for breast cancer imaging

Abstract

Research Objective and Relevance:

In an effort to speed up the translational activities that build a bridge between science and medicine, the National Institutes of Health has undertaken a series of initiatives known as the NIH Roadmap for Medical Research. “Molecular Imaging” is not only one of these seven initiatives but also a pivotal one for the others. Molecular imaging has a unique potential to play an important role in detection, diagnosis and even treatment of the disease.

Fluorescence imaging is currently the only and most sensitive “Optical Molecular Imaging” technique that can provide distribution of “Molecular Probes” in thick medium such as breast tissue. However, quantitative fluorescent imaging is very challenging. Although extensive effort has been spent in the last decade, there is only a single study reported in the literature for in vivo 3D fluorescence imaging of breast using ICG, which is the only FDA available optical contrast agent for human use. Moreover, the low-level fluorescence signal also makes the acquisition of in vivo data in a short time very demanding and hence limits the temporal resolution.

During her PhD study, Dr Lin, the fellow in this application, has mainly worked on developing a quantitative fluorescence imaging system for animal imaging. Her simulation and experimental studies all confirmed that multi-modality imaging was pivotal for quantitative fluorescence imaging. Indeed, outcome of her PhD study was first-of-its-kind hybrid x-ray computed tomography and fluorescence imaging system for animal imaging. Our main objective in this application is to translate this multi-modality approach into clinical settings. Mainly, she will apply her modeling and instrumentational expertise that she gained during her PhD to develop a hybrid dynamic contrast enhanced MR-fluorescence imaging system for breast cancer. The advantages of this system will be several-fold. First, the MRI counterpart will provide the standard clinical contrast enhancement data and detect the location of lesion. Using MR information, fluorescence imaging system will provide quantitatively accurate distribution of the contrast agent ICG in breast with high temporal resolution. Our hypothesis is that “the accurate enhancement kinetics of the MR and fluorescent agents obtained by this hybrid system will increase the overall specificity in differentiation of benign and malignant lesions”

Project Impact:

In 2007, the American Cancer Society updated its guidelines to recommend that women considered to be at high risk for developing breast cancer should get an annual breast MRI exam in addition to their yearly mammogram. Several studies have proven that the MRI detects malignant cancers which are occult on mammogram and ultrasound, and as such it has fast becoming the most popular imaging modality for screening young women. Dynamic contrast enhanced MRI (DCE-MRI) has evolved into a standard approach for detection and diagnosis of breast lesions in recent years. However, despite its high sensitivity, DCE-MRI also detects many benign lesions, which may lead to great anxiety to patients, unnecessary

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biopsy or over-treatment. Hence, there is a great need to improve the specificity of the DCE-MRI.

The system proposed in this study is a relatively low-cost system and can be integrated to the clinically available MR systems. Fluorescence imaging will work as an add-on to the existing MRI. The proposed study will be performed using a clinical 3T MR scanner. No additional scanning time is required for the optical data acquisition due to the simultaneous data acquisition scheme. The advent of such a multi-modality approach based on a high field MRI system with improved specificity in breast cancer detection would strengthen the role(s) of MR in breast cancer management. The simultaneous MRI-FDOT imaging modality proposed in this application is an innovative approach, and if successful, could improve the ability in better characterization of breast tumors.

Importance of the Research:

There is an ongoing effort to keep the radiation dose minimal and one of the best examples of these is a new law (AB 929) co-sponsored by the Breast Cancer Fund (CA) that creates quality standards to ensure that patients receive the lowest possible dose of radiation without compromising image quality. This novel multi-modality imaging platform will not employ ionizing radiation and we believe it will contribute to the ongoing advocacy efforts for safety of woman. The improved specificity would strengthen the role of this hybrid system in pre-operative staging, and also in screening of young women.

Due to low specificity of the DCE-MRI, the false positive findings usually results in the painful invasive procedures such as fine-needle aspirations or surgical biopsies. The objective of this research is to reduce these painful and unnecessary invasive procedures by improving the specificity of the DCE-MRI using additional information provided by the ICG enhancement kinetics. Cancer patients are expected to benefit from this technology development shortly after the completion of this proposed project due to the design of this proposed technology that is directly targeted for clinical studies. Besides this short-term impact, the developed system in this proposal will also have a great long-term potential to serve as an essential platform during the development and testing of molecular targeted agents on patients. Unfortunately, they are not approved for human use yet, but the first agents have been demonstrated in pre-clinical studies and their clinical availability is not a matter of if but when.

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PI Name: Michael Rosenfeld, PhD

Institution: University of California at San Diego

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: A strategy for the identification of tumor translocations in breast cancer

Abstract

Estrogen is essential for the physiological function and growth of the mammary gland as well as in the initiation and in the progression of breast cancer. The effect of estrogen is mediated by specific transcription factors, the estrogen receptors (ERs), that when activated regulate the expression of specific target genes.

While transcriptional regulation by estrogen receptor is well understood at a two-dimensional level, only recently have we realized that estrogen-dependent regulation of chromosomal organization in the three dimensional space of the nucleus, plays an important role both in transcriptional regulation and in chromosomal reorganization events. Thus, we found that estrogen can cause long-distant chromosomal interactions between regions on different chromosomes that could also be involved in breast cancer development. Moreover, we found that two estrogen target genes (TFF1 and GREB1) on independent chromosomes localized independently in the nucleus in the absence of E2; however, after estrogen-stimulation, the two genes now exhibit parallel physical proximity. These findings actually suggest that aberrant chromosomal rearrangements in breast cancer may require this ligand/ER⁺/PR induced proximity to exhibit translocation as a result. This possibility is suggested by the fact that abnormal chromosomal interactions or translocations have been reported in prostate cancer and in an uncommon form of breast cancer. Based on these observations, I hypothesize that tumor translocations are non-random events and that the ligand, by creating a specific physical contact between different regions in the genome, and recruitment genotoxic stress-induced enzymes that cause double stranded DNA breaks, underlies chromosomal translocation events in breast cancer. Therefore, the aims of this proposal are: (i) to identify specific translocation events in breast tumors by a deep sequencing approach and, (ii) to characterize the molecular mechanism involved in the formation of chromosomal translocation and link this to specific enzyme causing DSBs.

The discovery of translocation events in breast cancer could be a critical first step toward improving the prevention, diagnosis, and treatment of breast cancer. The proposed study in this application will provide quantitative, digital and real-time database information used for identification of new targets for cancer therapy. Additionally, it will provide new insights in breast cancer diagnostic, development and progression and additional strategies to modify therapeutic approaches to breast cancer.

Pending Execution of Grant Agreements

PI Name: Peter Laird, PhD

Institution: University of Southern California

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Multiplexed DNA methylation biomarkers as a diagnostic tool for the early detection of breast cancer.

Abstract

The explosive growth being witnessed in personalized medicine has yet to be harnessed in the early detection of breast cancer. Breast cancer is the second leading cause of cancer-related mortality in women with approximately 40,170 breast cancer deaths expected in 2009. The 5-year survival rate for patients with localized breast cancer is over 95% while that of metastatic cases is less than 30%, highlighting the importance of early detection and treatment of breast cancer. The early detection of breast cancer, using mammography, has been shown to reduce mortality in women with breast cancer. Despite the advances in screening technologies including mammography and MRI, early detection has not eliminated aggressive high-risk cancers. This could be in large part due to the inability of current image-based screening techniques to distinguish between low-risk indolent tumors and high-risk aggressive tumors resulting in the overtreatment and under-treatment of patients, respectively. Additionally, mammography is half as effective in detecting tumors in certain subsets of women including those who are young, Asian, on hormone replacement therapy, and/or have dense breasts. Women with dense breast tissue have been shown to be at increased breast cancer risk and failing to detect breast cancer early in these patients could be very detrimental. Hence, there is a need for an alternate screening tool that can detect high-risk patients earlier and with greater accuracy.

DNA methylation is the process by which certain cytosine residues in DNA can be modified by a methyl group to form 5-methylcytosine. Breast cancer progression is marked by increased levels of DNA methylation at certain sites in the human genome. DNA methylation that is present in the breast tumor can also be detected in the blood from these patients when tumor cell DNA is released into the bloodstream due to tumor cell death. Moreover, DNA methylation at specific sites differs between both breast tissue and blood from breast cancer patients compared to their normal counterparts. Our hypothesis is that a panel of biomarkers will be able to effectively distinguish between women with breast cancer and their normal/benign counterparts and hence be a valuable diagnostic tool in the early detection of breast cancer as well as in risk management. We propose to test this by DNA methylation profiling of breast cancer patients and their classification into distinct subgroups. Specific biomarkers that can effectively distinguish breast cancer patients from their healthy counterparts will be selected. The ability of these markers to monitor progression and/or recurrence in serially collected blood specimens from women with a full spectrum of breast disease will be tested. Lastly, the diagnostic performance of these markers will be tested in blood collected from cases vs. controls when compared to routine mammography.

The major advantage of our approach of using a DNA based diagnostic test over current screening tools like mammography is its potential ability to distinguish high-risk tumors based on the underlying biology of the tumor, an increased frequency at which the test could

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be administered, and its potential ability to detect tumors 6-12 months earlier than mammography.

The development of a minimally invasive and cost-effective diagnostic test for the early detection of breast cancer would clearly be of enormous benefit to breast cancer patients. Such a test could help alleviate some of the difficulties in determining which patients showing potentially suspicious lesions should be biopsied and would supplement and/or replace imaging-based screening in a subset of women. Due to the potential ability of our approach to distinguish high-risk tumors based on the underlying biology of the tumor, it could lead to greater reductions in breast cancer mortality. It could also reduce the over-treatment of patients at minimal risk, thereby greatly enhancing their quality of life. By identifying patients with the highest-risk and targeting preventive interventions for these patients, it will help us harness the power of personalized medicine.

Pending Execution of Grant Agreements

PI Name: Michael Clarke, MD

Institution: Stanford University

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Functional analysis of the Dlk1-Gtl2 imprinted region in breast stem cell differentiation and tumorigenesis

Abstract

Breast cancer continues to rob the women of their health, their productivity and their very lives, being the most common malignant disease in Western women. In 2009, an estimated 192,370 women will be diagnosed with breast cancer and 40,000 women will be lost to this disease only in U.S. Women with breast cancer have more treatment options and a better chance of long-term survival than ever before, in part, because much of the scientific research in breast cancer has focused on understanding the initiation and development of the disease. Even though, a more complete understanding of the normal mammary gland will be a critical underpinning of continued advances in detecting, preventing and treating breast cancer with therapies that spare normal tissue from therapy-related toxicity.

The current data suggests that like other stem cell containing organs such as the blood and the brain, breast development is also a hierarchical process that begins from mammary stem cells (now referred to MaSC). It has been demonstrated that the injection of one MaSC from a mouse is able to regenerate an entire functional mammary gland. In both human and mouse, the MaSC are unique cells found in a very low frequency in breast tissue. Their function is to develop and maintain the mammary gland tissue over a women's lifetime. This is because the MaSC are endowed with both the ability (1) to produce more mammary stem cells through a specialized division process called self-renewal and (2) to make the differentiated or specialized cell types found in the mammary gland. Therefore, MaSC are thought to be one of the most important cells that are responsible for maintaining the integrity of the mammary gland. Part of our project will consist of directly linking mammary gland development with breast cancer in order to identify which mammary epithelial cells are predisposed to oncogenic transformation.

MicroRNAs (or miRNAs) are small RNA molecules that have important roles for the proper function of a cell. Although miRNAs are encoded by a small percentage of genes, they are not translated into proteins like most of the rest of the RNA in a cell. Instead, they are processed into a special type of structure that interferes with the expression of specific target genes. One of the functions of certain miRNAs is to regulate self-renewal and differentiation in stem cells. Due to the important biological roles of miRNAs, their mutations or aberrant expression has been demonstrated to be directly associated with human cancers. One major aim of this proposal is to compare the levels of miRNAs in all known types of normal and cancerous breast cells. To that end, we have validated a highly sensitive technique geared towards the detection of miRNAs. Using this technique, we have analyzed the expression of 460 miRNAs in as few as 50 mouse normal and cancerous breast cells. This powerful technique will allow us to investigate the levels of miRNAs in primary patient tumors or biopsies where the major obstacle has been low cell number yield.

Nine percent of described miRNAs are clustered in a region of the genome called Dlk1-Gtl2. This genomic region is imprinted, meaning the miRNAs localized there are only expressed from the mother's copy of the gene. This region has also been identified as a cancer

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susceptibly locus in mice. Previous studies performed on human samples from primary tumors arising in different organs have shown that some of these clustered miRNAs have a tumor suppressor function (protects the cell from becoming a tumor-forming cell) and in ovarian cancer, the loss of their expression is associated with poor patient survival. However, not much is known about the protective effects of miRNAs in breast tumors. Therefore, we will investigate the functional role of the miRNAs expressed from the Dlk1-Gtl2 cluster in normal mammary development and assess how those miRNAs are perturbed in oncogenic transformation. We will also determine if the level of miRNA expression from the DLK1-GTL2 cluster have prognostic value relative to the outcome of the breast cancer patients. Our preliminary evidence shows that all the miRNAs localized in the Dlk1-Gtl2 cluster are highly expressed and are very abundant in mouse MaSC. In a well characterized experimental mouse model that develops spontaneous breast tumors (MMTV-Wnt-1), we observed that the same miRNAs are expressed at a lower level in breast cancer cells. Part of our project will consist of restoring the expression certain Dlk1-Gtl2 miRNAs in breast cancer cells to determine if their potential tumor suppressor functions can prevent tumor growth in vivo. The last part of the proposal will consist of understanding why certain cancer cells lose the expression of these miRNAs: we will investigate if the lower level of the miRNAs from the Dlk1-Gtl2 cluster is due to a mutation in the genomic sequence or a deregulation in their production processes. These studies have important implications for the introduction of miRNAs as direct therapeutic targets and/or treatment vehicles.

The work that we propose in this study may provide novel insights into (i) the functions of miRNAs from the Dlk1-Gtl2 genomic region in normal mammary gland development and tumorigenic transformation; (2) consideration of those miRNAs as possible prognostic markers and (3) miRNAs as therapeutic targets for the treatment of breast cancer. We believe that this project can help to understand the potent roles of miRNAs in cellular transformation. Furthermore, the combination of cutting edge technology and the research environment at Stanford is the perfect scientific environment to accomplish the described project.

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PI Name: Jessica Tyler, PhD

Institution: University of Colorado Health Sciences Center

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Using a novel epigenetic mark to provide fundamental insights into the biology of breast cancer

Abstract

Scientific objective and Rationale. 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 breast cancer. I am 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. This modification allows the histones to be removed from the DNA, to allow gene expression and DNA damage to occur more readily. Importantly, we 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.

The proposed studies will use cutting edge technologies to discover the exact locations on the chromosomes of these chemically modified histones that are so much more abundant in breast cancer cells. This in turn may explain the basis for the genomic instability and altered gene expression that causes breast cancer. I will also use new techniques to observe, literally, the ability of these chemically modified histones to move onto and off of the genetic material within breast cancer cells. This study will be the first of its kind for any cancer, and its findings are likely to represent a huge leap forward in our understanding of the fundamental basis of breast cancer progression.

Career goals. Because a close friend I know suffered from breast cancer, it is my personal goal to make fundamentally important contributions towards developing a cure for breast cancer. Given that we still don't really understand the fundamental basis for breast cancer, I decided that the way to make the most impact on this awful disease was to first learn to identify the important biological questions and how to design experiments to answer them. As such, training in the laboratory of a world-leading basic researcher, such as Dr. Tyler, was the obvious route to take, and then apply this knowledge to the study of breast cancer. I am confident that the rigorous research training coupled with the constant interactions that I have here with breast cancer researchers will uniquely prepare me to effectively answer critically important questions remaining in our understanding of breast carcinogenesis.

Ultimate applicability. To our knowledge, we are the first scientists to discover this chemical change in humans, not to mention that it is greatly elevated in all cancers, including 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 what this means for understanding

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breast cancer progression. As such, this project may reveal that this chemical change is a novel causative agent of breast cancer, and via our 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. As such, these studies could also result in not only the development of a kit for a new biomarker that predicts treatment outcome and prognosis of breast cancer, but also the development of therapeutics to reverse this specific chemical modification towards curing breast cancer. In addition to greatly advancing the field of research and our understanding of the fundamental causes of cancer, these studies could help patients with any cancer.

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PI Name: Tian Liu, PhD

Institution: Emory University, Winship Cancer Institute

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Early detection of lymphedema using ultrasound elastography

Abstract

“Lymphedema is a constant reminder of my cancer. You cannot really forget that you have had cancer because you are reminded everyday.” - Anonymous breast-cancer survivor

Arm lymphedema is a debilitating morbidity of breast-cancer treatment, affecting approximately 25% of breast-cancer survivors. Lymphedema can limit range of motion, cause pain or weakness, and may result in stiffness of the affected arm. Lymphedema profoundly affects the patients' quality of life.

Currently, lymphedema is clinically diagnosed by medical history review and physical examination that includes circumferential or volume measurement of the affected arm. Studies have shown the lack of consistency and rigor in these methods of measurement. Noninvasive and early detection of lymphedema is lacking but highly needed in the clinic, as early diagnosis precedes successful intervention.

We propose to develop a novel ultrasound device for lymphedema detection in breast-cancer treatment. Ultrasound is safe, noninvasive, cost-effective and widely accessible, making it well suited for clinical implementation. Our ultrasound technology provides accurate measurement of local tissue stiffness and therefore, may provide early diagnosis of mild and subclinical lymphedema. Early detection will enable early treatment and ultimately may improve many breast-cancer patients' quality of life.

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PI Name: Stephen Kron, PhD

Institution: University of Chicago

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Proteomic dissection of DNA damage response in breast cancer cells

Abstract

Breast cancer is one of the most common cancers among women in the United States. Radiation therapy is an important tool for breast cancer treatment; it is commonly used after surgery to reduce the chance of recurrence by targeting any remaining cancer cells, and to improve the long-term survival rate. In advanced breast cancer that has spread or has recurred, radiation therapy can increase life expectancy and quality of life. However, since radiation causes damage to both cancerous and normal tissues, its use needs to be carefully controlled. In order to improve the effectiveness of radiation therapy and to minimize normal tissue damage, it would be beneficial to sensitize cancer cells to radiation. Radiation causes cell death by damaging DNA to form breaks in the DNA strands. When cells detect DNA damage, they slow down their growth and repair damage, or undergo cell death if the damage is too severe. This process is called the DNA damage response. In order to understand how cancer cells respond to radiation, it is crucial to study proteins that participate in this process.

One protein that participates in DNA damage response, called histone H2AX, was found to be modified when cells are irradiated. It was later found that this H2AX modification can be used as an indicator of how much DNA damage cells carry. When cells are irradiated, the modified form of H2AX, called gamma-H2AX, can be detected as spots called ionizing radiation induced foci (IRIF), located on the DNA where it has been broken by radiation damage. As the cells repair their damage, the gamma-H2AX disappears. Many other DNA damage response proteins are also found together with gamma-H2AX in the foci, and this seems to be essential for the proper DNA damage response to take place. Thus, we hypothesize that factors that influences protein accumulation at gamma-H2AX foci and their disappearance after irradiation determines how sensitive the cells are to irradiation. In this proposal, we will develop a method to isolate proteins that associate with gamma-H2AX and damaged DNA in IRIF. Then we will compare isolated proteins from irradiated cancer cells at different time points. Since radiation therapy is currently given in daily fractions, we hope to understand the state of cancer cells after receiving one dose of radiation and then the state before getting a second does. Any changes in the protein profiles in early versus later time points will provide clues to understand determinants of cellular response to radiation therapy, and may potentially reveal novel targets that can be used to develop radiation-enhancing drugs. Once candidate proteins are identified, we will validate how they affect the overall DNA damage response in breast cancer cells. We also plan to compare isolated proteins from breast cancer cells that are treated with a drug called ABT-888, as this drug seem to change the appearance of IRIF in irradiated breast cancer cells at later time points. Since ABT-888 is currently in clinical trials for breast cancer treatment, it will be helpful to understand what is different about the IRIF proteins in the drug treated cells. These studies will provide a better understanding of cellular response to radiation, which can be used to design better schedules for radiation therapy to improve outcome. This work will particularly

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benefit patients with more advanced stages of breast cancer, with hope to improve the ability of radiation therapy to effect cures and not just to prolong life.

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PI Name: Charles Clevenger, MD, PhD

Institution: Northwestern University, Feinberg School of Medicine

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Cyclophilin B as a novel target for synthetic antigen binders in breast cancer

Abstract

Breast cancer is the most common cancer in women and is responsible for almost 20 percent of all cancer deaths in women. While some tumors respond to current therapy, there are still many subjects that do not respond. Therefore, new therapeutic targets in breast cancer cells are clearly needed. Cyclophilin B (CypB) is one member of the peptidyl prolyl isomerases family. Our laboratory has found that CypB is overexpressed in human breast cancer tissue and contributes to the expression of many genes involved in the development and progression of breast cancer. In addition, reduction of CypB levels was found to decrease the growth and invasive properties of breast cancer cells. Genetically-modified mice lacking CypB demonstrate delayed mammary growth and lactation. All of these data suggests that CypB plays a critical role in the development and progression of breast cancer and is a promising target for cancer therapy. However, no specific agent target CypB currently exists.

Synthetic antigen binders (SABs, also called synthetic antibody), are small, modified forms of antibody. They can be rapidly screened and genetically modified for optimal performance for therapeutic purposes. While normal antibodies only target on cell surface proteins, SABs can be engineered to target proteins that reside within the cells through a novel approach called receptor-mediated delivery with a peptide called substance P (SP). SP is a neuropeptide that rapidly enters into the cell upon interaction with its receptor on the cell surface. Attaching SABs to SP allows the SABs to efficiently entry into the cell and recognize their specific protein targets within the cell. Compared to traditional antibody, a major advantage of this approach is that it can penetrate live cells and directly target intracellular signaling pathways exploited by cancer. Given the critical role of CypB in breast cancer, we hypothesize that that anti-CypB SAB-SP can be used to specifically target CypB and inhibit its functions in highly controlled ways in breast cancer cells. Our overall objective of this proposal is to develop novel synthetic antibodies and validate their efficacy in breast cancer cells in vitro and in vivo.

In aim 1, we will generate SABs against CypB, conjugate them to SP, and then evaluate the delivery of anti-CypB SAB-SP conjugates into a panel of human breast cancer cell lines. The internalization of the anti-CypB SAB-SP antibodies into breast cancer cells will be assessed by both biochemical and microscopic approaches. After we have the anti-CypB SAB-SP in hand, we will test the effectiveness of anti-CypB SAB-SP at inhibiting cell growth, cell survival, and cell invasion in breast cancer cell lines in aim 2. We will then examine the efficacy of anti-CypB SAB-SP against human breast cancer in a mouse tumor model system in aim 3. We predict that anti-CypB SAB-SP will inhibit the breast cancer cell growth and metastasis in both cells and mouse model.

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Our study will allow us to develop SABs-SP to target human breast cancer and examine their efficacies in inhibiting cell proliferation and metastasis. The successful completion of our study may lead to a clinical trial using anti-CypB SAB-SP to treat breast cancer in the near future. Some highly significant breast cancer targets such as CypB reside within the cell, where they are inaccessible using traditional antibody strategies, which only bind to proteins on the surface of cells. The receptor-mediated delivery of synthetic antibody approach represents a paradigm shift in antibody therapy, because it has the potential to take the fight directly to the intracellular molecules. Our proposed studies will set the stage for the future translation of the use of these antibodies for therapeutic purposes.

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PI Name: Mary Hendrix, PhD

Institution: Children's Memorial Hospital

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Validating the embryonic morphogen Nodal as a new therapeutic target for breast carcinoma

Abstract

Public Abstract: One of the leading causes of death among women, breast cancer kills the majority of patients by complications of tumor recurrence and metastasis; however, most breast tumors don't develop initially with the ability to metastasize. At first, tumor cells require close contact with their neighbors in order to proliferate and grow in a localized tumor. As breast cancer progresses, tumor cells may become metastatic and acquire the ability to leave the original tumor, migrate to distant sites and establish new tumors. Interestingly, these features recapitulate several normal developmental processes involving stem cells. Our laboratory had shown that breast tumor cells secrete a protein, called Nodal, which is normally active only in stem cells and serves as a master regulator of the processes that allow stem cells to migrate and proliferate. This finding suggests that tumor cells may acquire metastatic potential by inappropriately reactivating processes that normally promote embryonic development. In normal development, however, Nodal has an inhibitor, termed Lefty, which is also produced by stem cells and serves to limit the Nodal-induced migration and proliferation of these cells. Breast cancer cells do not produce Lefty and therefore are able to respond to Nodal without regulation and to continue to divide and invade surrounding tissue. Our laboratory has shown that exposing breast cancer cells to Lefty is sufficient to inhibit their uncontrolled proliferation and migration and convert them to a less malignant state. This unexpected discovery suggests that cancer cells may be more plastic, or malleable in their fate choice, than previously believed and may have the ability to become more or less aggressive depending on the environmental cues received.

The current proposal aims to deepen our understanding of the function of Nodal in breast cancer progression and determine whether it may be a potential target for therapy. Similar to normal tissues, breast cancers are believed to harbor a small population of tumor stem cells which are responsible for tumor growth. It has been shown that breast tumor stem cells are the most invasive cells in a tumor, with the highest potential for proliferation and resistance to therapy. Tumor stem cells also represent the most likely mechanism by which tumors recur after treatment and spread to other organs. Therefore, determining the mechanisms by which breast tumor stem cells arise and the factors that contribute to their survival may improve our ability to treat the most devastating aspects of the disease. As Nodal is essential for embryonic stem cell function and the presence of Nodal is essential for maintaining tumor growth, the central hypothesis of our current proposal is that Nodal may facilitate the generation and maintenance of breast tumor stem cells, by endowing them with properties of proliferation and migration normally restricted to early development. If our hypothesis is correct, then blocking Nodal in breast cancer may be an effective way to target breast cancer stem cells and prevent metastases and recurrence of the disease in patients.

It is important to note, however, that in addition to Nodal there are other factors known to regulate stem cell self-renewal and plasticity that also participate in regulating cancer stem

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cells. Studies have shown that the interactions between some of these signaling pathways are as important as the function of any one factor alone, necessitating the need for combined studies of signaling pathways in tumor cells. One such regulator is TGF β , a member of the same superfamily as Nodal, which is strongly implicated in the development of breast cancer and has been shown to be essential for breast cancer stem cell function. As TGF β and Nodal activate cellular responses using some of the same molecules, part of our study will also include a careful investigation of the interaction between these two pathways and the ways in which they together modulate the behavior of breast cancer stem cells. Interestingly, while blocking the activity of TGF β has been shown to inhibit the invasive ability, but not the proliferation of breast cancer stem cells, interfering with Nodal function has directly impeded the proliferation of breast cancer cells, suggesting that combined inhibition of these factors, or blocking the intersection of their activities may provide a more potent therapeutic opportunity than targeting either factor alone. As increasing evidence suggests that breast cancer stem cells are responsible for the propagation and metastasis of breast cancer, improving our understanding of the pathways that sustain these cells may provide better opportunities to treat breast cancer patients.

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PI Name: Kathrin Kirsch, PhD

Institution: Boston University School of Medicine

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Development of inhibitors targeting p130Cas/BCAR1 family proteins

Abstract

Frequently, breast cancer is treated before and after surgery with chemotherapy, hormone and radiation therapies. Collectively, these treatments are called adjuvant therapies, which have increased women's survival from breast cancer. However, breast cancers can evolve and stop responding to chemotherapeutic drugs, including adriamycin, and hormone therapy with tamoxifen. A new generation of targeted biological agents demonstrates a high effectiveness at lower toxicity. However, treatment with these specific drugs is limited to the subset of breast cancers that overexpress the target, and eventually patients develop resistance to these drugs as well. This strongly indicates the need to develop novel approaches to fight breast tumor cells and to prevent or reduce drug-resistance. One protein implicated with tamoxifen as well as adriamycin resistance in mammary tumors is called p130Cas/BCAR1. p130Cas acts as a scaffold to integrate protein complexes that are involved in important mechanisms, which are often altered in breast cancer. Importantly, elevated levels of p130Cas characterize aggressive breast cancers. These characteristics indicate that drugs targeting p130Cas might have a high potential to fight multiple subtypes of breast cancer. However, to date no therapeutic agents directed against this protein have been developed and to our knowledge, we are the first to address this issue. We have established a novel decoy approach to block certain functions of p130Cas. Our recent studies indicate that this strategy can inhibit mechanisms that contribute to the malignancy of mammary adenocarcinomas whereas normal mammary gland development is not influenced. Importantly, this approach re-sensitized breast cancer cells to tamoxifen treatment. Here, we propose to develop and refine our approach to fight breast cancer by developing a small molecule to target p130Cas. We will refine our decoy system and perform in vivo experiments in mice as a basis for the subsequent production of a small inhibitor to potentially treat breast cancer in humans. Furthermore, we will investigate our hypothesis that this approach might enhance the effectiveness of adjuvant therapies in breast cancer cells.

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PI Name: David Frank, PhD

Institution: Dana-Farber Cancer Institute

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Identification of a new STAT3 inhibitor for breast cancer therapy:
Mechanistic and preclinical evaluation

Abstract

Breast cancer is the second leading cause of cancer deaths in women in the United States. In approximately 70% of these breast cancers, there is inappropriate activation of the protein STAT3, which promotes growth and survival of the breast cancer cells. We identified a compound, ST3-01, that inhibits the function of STAT3, and breast cancer cells treated with ST3-01 die. Our hypothesis is that this compound, by being a direct STAT3 inhibitor, may be useful for the treatment of breast cancer. In this proposal, we will determine how ST3-01 blocks STAT3 from functioning, if this compound kills breast tumor cells in mice, and what drugs can be combined with ST3-01 to create a more personalized, less toxic form of therapy.

The knowledge of how ST3-01 blocks STAT3 is critical for optimizing the effectiveness of this compound, and may make it more likely to be used as therapy. In addition, determining if ST3-01 works in mice provides important information that can be used when designing clinical trials with this drug. Finally, if something is wrong with a normal cell, it receives a signal to die. However, cancer cells have strategies to resist these signals. One strategy that many cancer cells use to stop these signals is to increase the number of survival proteins. This makes these cancer cells very dependent on these survival proteins: if there is a decrease in number of these proteins, the cells die. There are techniques to identify which of these survival proteins are preventing the tumor cells from dying. Moreover, there are new drugs that directly affect the function of these survival proteins, and they are currently in clinical trials for a number of cancers. Combining these drugs with our new compound that inhibits STAT3 has the potential to very specifically target these cancer cells, while having little effect on normal cells. Taken together, the work that I propose for this fellowship has the potential to greatly impact future breast cancer therapy.

The work in this proposal deals with a compound that has the potential of being a future drug used for breast cancer. Understanding how it functions and if it works in animals are key steps to bringing ST3-01 closer to the possibility of future use as therapy. In addition, understanding how combinations of existing or new chemotherapy works with ST3-01 could greatly enhance the efficacy of treatment, providing a more targeted form of therapy. Therefore, the work in this proposal has the potential to have great impact on patients with breast cancer.

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PI Name: Robert Damato, PhD

Institution: Children's Hospital, Boston

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Inhibition of Tumor Endothelial Marker 8 (TEM-8) as an anti-angiogenic therapeutic approach for breast cancer

Abstract

The American Cancer Society estimates that there will be 192,370 new breast cancer cases and 40,170 deaths from breast cancer in 2009 in the United States. It is now well established that, in common with other solid tumors, breast carcinoma growth is dependent on the process of new blood vessel growth or 'angiogenesis'. Drugs which interfere with new blood vessel growth prevent tumors from growing beyond a certain size and can also shrink tumors in some cases. A number of these angiogenesis inhibitors have been developed and are used as part of cancer therapy regimens.

Currently available angiogenesis inhibitors typically target one molecule that is known to have an important role in blood vessel growth. This molecule is a growth factor for endothelial cells called vascular endothelial growth factor (VEGF). Drugs that target VEGF are effective for inhibiting the growth of some types of tumors. However in breast cancer a number of different growth factors, and not just VEGF alone, are present at high levels and are involved in stimulating the growth of new tumor blood vessels. This makes the development of effective anti-angiogenic drugs for breast cancer particularly challenging, necessitating the identification of additional anti-angiogenic targets and therapeutics. My long-term objective is to identify new targets implicated in the angiogenic cascade with the goal of generating improved and more broadly efficacious anti-angiogenic therapies for breast cancer, thereby preventing blood vessel growth that contributes to the catastrophic growth of tumors.

The current project involves an investigation of a molecule called tumor endothelial marker 8 (TEM-8) as a therapeutic drug target in breast cancer. A number of different lines of evidence point to this factor as being central to blood vessel growth within tumors. TEM-8 is known to be present in the blood vessels within tumors, but not in normal non-tumor vessels present in the rest of our body. TEM-8 has been shown to interact with proteins termed 'extracellular matrix proteins' that have a crucial role in angiogenesis, promoting the formation of new vessels in tumors. Importantly, exciting results from our laboratory have shown that a compound that blocks TEM-8 on cells lining blood vessels can inhibit angiogenesis and tumorigenesis in a lung cancer model. Crucially, it has been shown that TEM-8 is expressed on the blood vessels within tumors of the breast. The fact that this molecule is not expressed on normal blood vessels is also important, as it indicates that drugs that specifically and selectively target TEM-8 for the treatment of breast cancer would not be expected to have major side effects. Almost all drugs have side effects, and these often relate to their activity at parts of the body other than where they are intended, in this case the tumor endothelium. Side effects of VEGF inhibitors, including hypertension and proteinuria in systemic cancer therapy limit the doses that can be given and present major difficulties for cancer patients.

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I hypothesise that TEM-8 plays an important role in breast cancer angiogenesis and that therapeutics that inhibit TEM-8 will inhibit breast tumor growth. I will first identify therapeutic compounds and proteins that bind to TEM-8 and inhibit its activity. I expect to demonstrate that these TEM-8 inhibitors can inhibit blood vessel growth firstly in a cell culture system, and later in mice. I will then investigate whether effective anti-angiogenic compounds which inhibit TEM-8 can also inhibit breast tumor angiogenesis and the growth of breast carcinoma in mouse models. I will also investigate whether these drugs can prevent the spread of breast cancer cells to other organs in the body, a destructive process known as 'metastasis' which is the cause of the majority of deaths from breast cancer.

TEM-8 is known to be present in the blood vessels within breast tumors and is thought to be important in angiogenesis, but the effect of therapeutically blocking this molecule on breast cancer progression and metastasis has not yet been studied. This project will advance our understanding of the role of TEM-8 in breast cancer, simultaneously generating TEM-8 inhibitors that could form the basis of new anti-angiogenic therapy for breast cancer. More effective anti-angiogenic and anti-metastatic drugs for breast cancer would result in reduced morbidity and mortality for these patients. Currently available anti-angiogenic drugs, as part of multi-drug approaches, have been shown to extend survival of other cancer patients including metastatic colon cancer and mesothelioma patients.

Targeting tumor endothelial marker 8 with new therapeutic compounds is likely to represent an addition to the VEGF-targeting strategy currently employed in breast cancer. I hypothesise that TEM-8 functions to regulate angiogenesis stimulated by VEGF, and crucially by other growth factors that are present in breast tumors. Thus, therapeutic compounds that inhibit TEM-8 would be expected to offer additional anti-angiogenic benefits for breast cancer when added to anti-angiogenic drugs targeting a single growth factor, such as the VEGF inhibitors. The development of efficacious anti-angiogenic, anti-tumorigenic and anti-metastatic therapies which prolong survival and have minimal side effects is of extremely high importance for the treatment of breast cancer patients. I intend to demonstrate that inhibition of TEM-8 may represent an effective strategy for this critical goal.

Pending Execution of Grant Agreements

PI Name: Kornelia Polyak, MD, PhD

Institution: Dana-Farber Cancer Institute

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Molecular pathways in myoepithelial cell differentiation: role in breast tumor progression

Abstract

Ductal carcinoma in situ (DCIS) is believed to be a precursor of invasive breast carcinomas. It accounts for 15-25% of newly diagnosed breast cancer cases in the United States. Detection of breast cancer at the DCIS stage greatly reduces breast cancer-related morbidity and mortality, since DCIS is essentially a surgically curable disease. However, a significant fraction of DCIS patients develop recurrences and progress to invasive disease. Despite the clinical importance of this issue, we are still unable to predict which DCIS patients will progress to invasive disease and more importantly, how to prevent this progression.

The normal mammary milk duct is composed of an inner luminal epithelial cell layer surrounded by myoepithelial cells that help expel milk during lactation due to their contractile function. It is believed that luminal epithelial and myoepithelial cells develop from mammary epithelial stem cells. During breast tumor progression myoepithelial cells become progressively altered as DCIS-associated and normal myoepithelial cells have distinct properties and their number also decreases. An important hallmark of invasive breast cancer is the focal disruption or complete disappearance of the myoepithelial cell layer. However, the mechanisms that lead to alterations in myoepithelial cells and their eventual loss are almost completely unknown.

In this application, I will dissect the basic biology of myoepithelial cell differentiation and how abnormalities in this process occur in DCIS leading to the loss of this cell layer and subsequent invasion. I will accomplish this aim by isolating and characterizing myoepithelial precursors and differentiated myoepithelial cells from normal human breast tissue and different types of DCIS tumors. By comprehensively analyzing the molecular profiles and functional properties of the isolated cells, I will identify genes and signaling pathways involved in myoepithelial cell differentiation and their abnormalities in DCIS. The proposed research will lead to the identification of molecular markers that can be used for risk prediction in patients diagnosed with DCIS and yield new targets for cancer therapeutic and preventative approaches. Thus, the proposed research is highly clinically significant and will benefit patients diagnosed with DCIS and healthy women who are at high risk of developing invasive breast cancer.

Pending Execution of Grant Agreements

PI Name: David Pellman, PhD

Institution: Dana-Farber Cancer Institute

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Targeting centrosome clustering as a therapeutic strategy in breast cancer

Abstract

The goal of cancer therapy is to identify differences between normal cells and cancer cells, enabling selective killing of cancer cells. A well-known biological difference between normal and cancer cells is the number of cellular structures called, centrosomes. Centrosomes are organizing centers for the machine that distributes chromosomes properly when a cell divides into two daughter cells. Centrosome duplication is tightly controlled, and as a result normal cells possess only two centrosomes during the time when the chromosomes are being distributed to the daughters. By contrast, cancer cells commonly contain too many centrosomes. This is well-documented feature of breast cancer that correlates with aggressive behavior of tumors and also occurs in other solid tumors and some leukemias. My goal is to understand why cancer cells often have too many centrosomes and to determine if this is an Achilles' heel that can be exploited for therapeutic attack. We postulate that there may be a tug of war-- the extra centrosomes provide some initial growth advantage to the developing cancer cell, but this advantage is only realized if the cancer cell can control the potentially destructive aspects of harboring too many centrosomes. Our laboratory has recently used functional genomics and imaging to define the mechanisms used by cancer cells to control the destructive potential of extra centrosomes. One protein identified by this approach is an appealing drug target: this protein, HSET, allows cancer cells to organize extra centrosomes so that they are not harmful during cell division. Furthermore, we have evidence that inhibition of HSET can selectively kill cancer cells and not harm normal cells. Our initial findings lead to questions of why cancer cells have extra centrosomes in the first place. Do they provide an advantage? If so, what is the nature of the advantage? Would other defects be revealed if extra centrosomes are stripped away by HSET inhibition? Determining the answer to these questions might enable us to develop complimentary therapies that would synergize with HSET inhibition. In this proposal, I will first determine if extra centrosomes provide growth advantage to cancer cells in cell culture system as well as live animals by 'stripping' cancer cells of their extra centrosomes and monitor their growth and tumor initiation ability. Second, I will evaluate a potential approach to breast cancer therapeutics by HSET depletion in animals possess breast cancer. I will inhibit HSET before or after breast cancer formation in mutant mice prone to develop breast cancer and record the incidence of tumor initiation as well as progression/regression of pre-existing tumors.

Pending Execution of Grant Agreements

PI Name: Alan D'Andrea, PhD

Institution: Dana-Farber Cancer Institute

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Fanconi anemia/BRCA pathway and PARP1 inhibition in BRCA1-proficient breast cancers

Abstract

Breast cancer is a malignant tumor of the breast. Conventional breast cancer therapy (radiation and chemotherapy) can kill these breast tumor cells by causing DNA damage. Breast tumors can become resistant to these conventional therapies by developing and upregulating DNA repair mechanisms which repair the DNA damage. Our laboratory has identified and characterized a DNA repair pathway, called the Fanconi Anemia/BRCA pathway, which allows breast tumor cells to repair DNA damage. More recently, we have characterized a novel class of drugs which can inhibit the FA/BRCA pathway. We propose that these new drugs (FA/BRCA pathway inhibitors) can be used to block DNA repair in human breast tumors and to enhance the activity of conventional breast cancer therapy. We plan to determine whether these new FA/BRCA pathway inhibitors can enhance the activity of a new class of DNA repair inhibitors, called PARP inhibitors, when these two inhibitory drug classes are used in combination.

Pending Execution of Grant Agreements

PI Name: Alan Rigby, PhD

Institution: Beth Israel Deaconess Medical Center

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: In silico discovery and optimization of a novel estrogen receptor alpha antagonist degradation platform for the treatment of drug resistant breast cancer

Abstract

Breast cancer (BrCa) is the most common female cancer in the United States. The lifetime probability of developing BrCa is approximately 1 in 6. BrCa incidence rises steeply with age until age 45-50 when the rise is less steep, most likely due to changes in hormonal status. Increased estrogen levels are associated with increased risk for BrCa. The majority of BrCa is detected by mammography. Approximately 10% of patients have a positive family history of cancer. Despite some improvements in diagnosis and treatment BrCa remains a major cause of morbidity and mortality for women. It is estimated that ~210,000 women will be diagnosed with BrCa and ~41,000 women will die from the disease this year. Due to the fact that many patients diagnosed with BrCa do not fully respond to conventional therapy, and ultimately die from the disease, there remains a significant unmet need for novel therapeutic compounds.

The estrogen receptor (ER) and more specifically the alpha isoform (ERalpha) regulate cellular growth, proliferation, and differentiation. In addition to having prognostic value, ER is the most important biologic marker of therapeutic response in breast cancer. ERalpha protein levels are over expressed in drug resistant BrCa supporting a causative as well as prognostic-link to BrCa disease severity, which further articulates the importance of ERalpha as a therapeutic target in this disease. While SERMs including Tamoxifen and/or Raloxifene have been used to treat ER1alpha-positive BrCa patients, we believe that a considerable unmet need to develop novel and or more efficacious compounds that work through a unique mechanism that involves ERalpha degradation are desperately needed. The many patients treated with current anti-estrogen regimes that progress to develop resistance to these anti-estrogens resulting in a more aggressive, drug resistant BrCa, further support the need for novel therapies.

The proposed project is highly translational, driven by our attempts to discover transcriptional therapies that will be evaluated and subsequently optimized through in vitro and in vivo experiments of BrCa. The nuclear receptor family of transcription factors and more specifically the ER are established regulators requisite for a variety of biological processes, including growth, differentiation and development. This receptor also plays a central role in regulating pathological processes such as inflammation and cancer. Using our computer aided drug discovery (CADD) screening platform we have proposed to screen large publicly accessible chemical repositories for small molecule "hits" that target this well-defined molecular target. These small molecules would be capable of regulating aberrant gene transcription by removing the ER from the cell through proteosomal degradation would offer new hope in BrCa therapy.

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Using the combined expertise in computer aided drug discovery and structural biology in the Rigby laboratory and the considerable nuclear hormone receptor (NR) expertise in the laboratories of our collaborators; Dr Steven Balk at Beth Israel Deaconess Medical Center (Androgen Receptor) and Dr. Myles Brown at the Dana-Farber Cancer Institute (Estrogen Receptor) we propose to identify novel ER1alpha antagonist chemotypes using an iterative discovery program that we have developed and validated for in the nuclear hormone receptor super family space. Our studies in the prostate cancer field, developing a robust platform of small molecule androgen receptor (AR) degraders support the promise of this approach and the compounds that are identified/developed through its implementation. We believe that this initiative partners core competencies of the PIs including: in silico screening, quantitative structure activity relationship (QSAR) and computational combinatorial chemistry with ER molecular and cellular biology as well as BrCa pathology. The primary goal of this translational proposal that partner's computational/biophysical approaches with in vitro/ in vivo evaluation of small molecule "hits" is the discovery and development of novel small molecule therapies for the treatment of BrCa.

Pending Execution of Grant Agreements

PI Name: Zaver Bhujwala, PhD

Institution: Johns Hopkins University, School of Medicine

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: The role of HOXB13 in the development of tamoxifen resistance

Abstract

Tamoxifen is the most commonly used treatment for postmenopausal women with early stage estrogen receptor-alpha (ER) positive breast cancer. It is safe and quite effective, but initially responsive breast tumors often develop resistance and ultimately recur after many years.

It is very important to have an in depth understanding of the mechanism of tamoxifen resistance. Therefore, this research opportunity will give provide me training in various research techniques in breast cancer research. In addition, it will allow me to achieve skills for designing and performing experiments. In the future, the proposed experiment will provide me with a great experience as an independent breast cancer researcher. I will receive training in the laboratory of Dr. Zaver Bhujwala in the Department of Radiology of The Johns Hopkins University School of Medicine who is a world renowned researcher in breast cancer.

Research Plan: To investigate tamoxifen resistance in ER positive breast cancer, I will focus on an important pathway in breast cancer- the EGFR/HER and its possible regulator- a very important protein called HOXB7 protein. It is well known in the endocrinology field that increased levels of ErbB/HER family members can directly change the way the cell responds to tamoxifen. Our lab found that HOXB13 expression is significantly elevated in both primary cancer and distant metastasis and is involved in invasion of breast cancer.

Hypothesis: I propose that the HOXB13 is a putative regulator of the HER2 and ER alpha pathways and can be a key protein in the initiation and maintenance of tamoxifen resistance in breast cancers. By understanding these pathways, we will be able to identify key points in the signaling pathways and develop targeted drugs for SERM-resistant breast cancer.

Study design: I will investigate (1) if HOXB13 expression regulates HER2/neu expression in multiple breast cancer cell lines. (2) Investigate if HOXB13 acts as an enhancer, rather than a direct transcriptional activator. It was reported that in tamoxifen resistance, HER2/neu expression is associated with ER? and co-regulators such as AIB1 (SRC-3) and PAX-2. I will test if HOXB13 can alter the activity of the ER? co-regulatory molecules and how. (3) To gain insight globally and into the other targets of HOXB13 in the HER2/neu and ER-alpha pathway, I will perform CHIP-sequencing analysis. (4) Since HOXB13 is subject to epigenetic modulation, I will determine the role of methylation and chromatin modifications through the Polycomb group and Trithorax complexes in regulating HOXB13 expression. (5) Once identified, I will target critical nodes of the Her2/neu and ER-alpha pathway in tamoxifen-resistant breast cancer cell lines grown as xenografts, and assess therapeutic response using non-invasive in vivo imaging, biochemical and immunohistochemical techniques.

Applicability of the research: The proposed studies should contribute significantly to our understanding of how tamoxifen resistance in ER alpha-positive breast cancer develops. This study will help hormone receptor-positive primary breast cancer patients who are treated with tamoxifen-monotherapy. Potentially, drugs which can block HOXB7 function will reduce tamoxifen resistance by decreasing of EGFR and ER signaling pathway. Once these

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drugs are found to be safe for use, with low toxicity, they can be taken into Phase I/II trials by our oncologists.

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PI Name: Vered Stearns, MD

Institution: Johns Hopkins University, Kimmel Cancer Center

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Phase II study of 5-azacytidine and entinostat (MS-275) in patients with advanced breast cancer

Abstract

With advances in recent years in both the detection and treatment of breast cancer, the majority of women with early breast cancer will survive their disease. However, this is not the case for most women who develop a recurrence or are diagnosed with advanced disease. New treatment strategies are therefore urgently required in an effort to replace or improve the efficacy of chemotherapy and hormonal therapy.

Cancer may develop as a result of changes in a patients' genetic make-up. Recent research indicates that certain chemical changes that occur at the sites where genes are switched on or off may also lead to cancer development and growth. These "epigenetic changes" may include DNA methylation and can result in the altered expression of genes. New drugs which specifically target these epigenetic alterations represent an active and attractive area of new drug investigation and include the DNA methyltransferase (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors.

Laboratory studies have found that these agents can not only improve the effectiveness of standard chemotherapy but also slow the growth of breast cancer cells. The combination of a DNMT and an HDAC inhibitor can switch genes on that have previously been switched off, such as the estrogen receptor (ER), far more effectively than either agent alone. This has been shown to restore the effectiveness of tamoxifen where resistance to the drug had developed. The promise of these agents in the laboratory setting has resulted in the opening of a number of clinical trials at Johns Hopkins assessing the combination of a DNMT (5-Azacytidine, 5-AZA) and an HDAC inhibitor (entinostat, MS275) in patients with myelodysplastic syndrome, lung cancer and colorectal cancer. Preliminary results indicate that this combination is safe and tolerable and associated with improved outcome for certain patients.

We have designed a clinical trial in which men and women with advanced breast cancer will be treated with the combination of 5-AZA and entinostat. Tumor samples will be used to determine if reversal of epigenetic changes occurs with this combination of agents. Our hypothesis is that well-tolerated doses of 5-AZA and entinostat will result in breast cancer control or shrinkage (clinical response) in patients with advanced breast cancer. By undertaking laboratory studies investigating gene methylation and expression, we aim to not only gain an understanding of the way breast cancer develops and progresses but also of the mechanism of action of these agents. The proposed investigations will set the stage for future large trials in which agents that reverse epigenetic changes may be used in conjunction with or even replace chemotherapy in women with early and advanced breast cancer. We envision, therefore, that our work has a strong potential to reduce breast cancer recurrence and death rates within a decade.

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The study represents one step in a series of laboratory and clinical investigations that have been funded through the Stand Up To Cancer grant. The grant will support the efforts of Dr. Connolly on this project. The work described in this proposal and the direction of an experienced team of researchers will help Dr. Connolly learn the fundamentals of clinical investigation and translational research.

Pending Execution of Grant Agreements

PI Name: Amy Fulton, PhD

Institution: University of Maryland School of Medicine

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Prostaglandin E EP1 receptor in breast cancer metastasis and disparities

Abstract

Our laboratory is seeking to identify the mechanisms by which breast cancer cells spread to distant sites and to block these mechanisms. The ultimate goal is to reduce breast cancer mortality by preventing metastasis. Cyclooxygenase-2 (COX-2) is an enzyme that can cause breast cancer and contribute to metastasis when it is highly active. Drugs that inhibit COX-2 have been shown, in mice, to block growth and metastasis; however, COX-2 inhibitors, such as Celebrex and Vioxx, can cause heart problems. Therefore we are developing new therapeutic strategies in order to target COX-2 and other enzymes associated with it. The COX-2 enzyme facilitates the production of a family of lipids called prostaglandins. The primary prostaglandin produced by tumors is prostaglandin E2 (PGE2). High levels of PGE2 can promote the growth and spread of breast tumors by many mechanisms. PGE2 produces responses by binding to four different receptors, EP1, EP2, EP3 or EP4, on the surface of cells that triggers a cascade of events inside of the cell. We hypothesized that by blocking only the actions of PGE2 while not disturbing the entire COX-2 pathway, we might improve safety and therapeutic benefits. These four receptors can trigger a different response in the cell. Recently, there has been a paradigm shift in our understanding regarding the location and function of EP receptors. Studies have shown that these receptors are not only located on the surface of cells; but, also within or on the surface of the nucleus and that these nuclear receptors may trigger different events in the cell compared to those found on the surface. By analyzing tissue from human breast tumors, we and others have found that EP1 is found in the nucleus of cells and that little to no EP1 located within the nucleus is associated with worse survival. Additionally, we have also found that breast tumors from African American women, who frequently present with a more aggressive disease, have less EP1 compared to breast tumors from Caucasian women.

Using a mouse model of metastatic breast cancer, we have found that blocking the expression of the EP1 gene or blocking the action of EP1 with drugs leads to an increase in metastasis which suggests that EP1 is a protective receptor against metastasis. We will test the hypothesis that increasing the expression of EP1 through the use of genetic and pharmacologic mechanisms will reduce breast cancer metastasis. Our proposed studies will determine how EP1 suppresses metastasis by studying if the receptor from the cell surface functions differently than the receptor found on the nucleus and what genes are modulated. Studies from our lab have shown that epigenetics may play a role in determining how the EP1 gene is expressed. Epigenetics refers to changes, that can be inherited, which affect the expression of a gene without changing the DNA sequence. Preliminary studies have indicated that drugs which can affect the epigenetic control of a gene can increase the expression of EP1. Therefore, we will test if EP1 is regulated by epigenetic mechanisms and test if a drug, that is already approved for clinical use for myelodysplasia, can increase the expression of this protective receptor EP1 and suppress metastasis.

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Since mortality from breast cancer is generally due to metastasis, identifying factors that can decrease this process will reduce mortality. Our preclinical studies use a mouse model of triple negative (negative for estrogen, progesterone, and Her-2/neu receptors), late stage breast cancer which is difficult to treat and an overrepresented breast cancer subtype in African American women. Additionally, EP1 is expressed less frequently in breast tumors from African American women. If our hypotheses are correct we will demonstrate that therapeutic intervention, that has already proven to be effective in patients with hematological cancer, can increase EP1 expression and suppress metastasis therein reducing breast cancer disparities as well as improve the clinical outcome for all breast cancer patients.

Pending Execution of Grant Agreements

PI Name: Angelika Burger, PhD

Institution: Wayne State University

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Evaluation of aldehyde dehydrogenase inhibitors for the prevention and treatment of triple negative breast cancer

Abstract

Triple negative breast cancers are devoid of growth factor receptors (estrogen receptor, progesterone receptor, and HER2/Neu) against which we have effective and targeted therapies available that cause only mild side effects in breast cancer patients eligible for the treatments. The only option for triple negative breast cancer patients with either localized or advanced disease at present is cytotoxic chemotherapy. Approximately 15-20% of all women diagnosed in the US with breast cancer will have triple negative disease and the majority of these patients will be young women in their most productive ages. Triple-negative breast cancers are known to take a very aggressive course, and even if successfully treated after initial diagnosis, they have a very high rate of recurrence. Hence, there is a pressing need for the development of better therapies for the treatment of triple negative breast cancer patients.

It has recently been described that estrogen receptor negative, BRCA1-deficient breast cancer cells have an increased activity in the enzyme aldehyde dehydrogenase 1 (ALDH1). ALDH1 is also a marker of breast cancer stem cells, a rare cell fraction within a tumor that is resistant to cytotoxic chemotherapy and responsible for disease relapse. ALDH1 has been shown as a predictor of poor clinical outcome.

STUDY HYPOTHESIS. We have found that inhibitors of ALDH1 can reduce enzyme activity in the human triple negative breast cancer cells MCF10DCIS.com and as a result, their growth in stem cell assays. MCF10DCIS.com cells when grafted into mice will first develop ductal carcinoma in situ (DCIS), a less dangerous form of breast cancer, and then progress into invasive ductal carcinomas of the triple negative phenotype. After DCIS disease is occurring, the stem cell marker ALDH1 becomes upregulated and later in invasive cancers, pockets of highly ALDH-positive cells are seen. We hypothesize that the ALDH1 inhibitor disulfiram and its novel analog ANFD24 will be useful to prevent the development of invasive triple negative breast cancer in the MCF10DCIS.com mouse model, based on their ability to inhibit ALDH1 and breast cancer stem cell growth. We further hypothesize that the inhibition of ALDH1 will enhance the effects of cytotoxic chemotherapy such as adriamycin, paclitaxel and cyclophosphamide.

The hypotheses will be tested in three specific aims. First, we will use in vitro cultures of a panel of 7 estrogen receptor positive, HER2 positive and triple negative breast cancer cell lines respectively and test the ALDH1 inhibitors for their anti-proliferative effects on breast cancer stem cells as well as the bulk tumor cell mass in specialized assays that are established in our laboratory. We will further assess the impact of ALDH1 inhibition on stem cell properties such as self-renewal and survival. Second, we will use the MCF10DCIS.com mouse model to evaluate the efficacy of ALDH inhibitors in the prevention of the progression of DCIS to invasive cancer and that of tumor growth in invasive breast cancers. Third, in cell

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lines and the animal model we will test whether ALDH1 inhibition can enhance the activity of cytotoxic chemotherapy in drug combination experiments.

UNIQUE IMPACT ON THE UNDERSTANDING OF BREAST CANCER. The enzyme ALDH1 has emerged as a marker of poor prognosis in breast cancer and as a breast stem cell marker, yet it has not been exploited as a target for the treatment of breast cancer. Triple negative breast cancers lack “good” therapeutic targets such as the estrogen and HER2 receptors. Drugs that can inhibit a specific breast cancer-associated enzyme are very promising. This project will uniquely advance our understanding of whether ALDH1 is a valid target for the treatment of breast cancer and whether it can eradicate ALDH-positive breast cancer stem cells through the use of ALDH1 inhibitors. The latter e.g. disulfiram are known to be well tolerated in mice and humans. In fact disulfiram is a Food and Drug Administration approved drug for the treatment of alcoholism. Therefore, our studies with disulfiram and its analog as well as combinations with the standard therapies cyclophosphamide, paclitaxel and epirubicin /doxorubicin could be rapidly translated into clinical benefit for patients with triple negative breast cancer and lead to reduction in mortality over the next 5 years.

IMPORTANCE OF THE RESEARCH TO PATIENTS WITH BREAST CANCER.

Triple negative breast cancer will affect young women and in particular young African American women. Women that are diagnosed with triple negative breast cancer have a very poor 5-year survival, because the disease is very aggressive and requires systemic chemotherapy. Specific guidelines how to treat triple negative breast cancers do not exist. Hence, the development of novel drugs and better treatment strategies remains the biggest unmet need in breast cancer today.

Pending Execution of Grant Agreements

PI Name: Shana Sturla, PhD

Institution: University of Minnesota at Twin Cities

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Breast cancer therapy by tumor-specific inhibition of DNA-repair proteins

Abstract

Conventional chemotherapy drugs act by damaging DNA in tumor cells to trigger cell death. While there is modern medical concern that they are not specifically targeted therapies, it must be remembered that conventional chemotherapy has provided substantial benefit for women with triple negative breast cancers, and that improvements in breast cancer outcome over the past 20 years can be directly attributed to the wider use of chemotherapy in the adjuvant setting. Thus, despite certain drawbacks DNA alkylation therapy is effective in practice for many critical cases and remains a mainstay of combinations with newer tumor-specific and targeted therapeutics.

The process of DNA repair involves the enzyme-mediated removal of drug-induced DNA damage. Effective DNA repair activity is important for the survival of normal cells, but fast and efficient DNA repair in cancerous cells contributes to poor drug response or resistance. In breast cancer cells, DNA repair represent attractive and potentially effective therapeutic target. In the case of women with BRCA deficient tumors, we've now learned that indirect targeting DNA repair, with drugs like a PARP inhibitor shows high potential and could be considered a targeted therapy for this class of tumors. However, direct tumor specific DNA repair inhibition has not yet been attempted and represents an attractive approach to breast cancer therapy. In pursuit of tumor-specific DNA repair inhibition we propose development of multi-functional tumor-specific drugs that bind DNA and inhibit a specific cellular repair enzyme(s).

Irofulven is a unique DNA-damaging agent that has been established to be preferentially activated in cancer cells. This property, in addition to its high potency, has advanced irofulven into clinical trials for ovarian cancer (phase III), recurrent epithelial ovarian and peritoneal cancer (phase II), metastatic lung carcinoma (phase I), and acute leukemia (phase I). Further data suggests that irofulven-DNA adducts are recognized and repaired specifically by transcription-coupled nuclear excision repair (TCR), and high irofulven activity in lung, head, neck, colon and ovarian cancer cells correlates with TCR deficiency detected in these cells. Breast cancer cells, however, are only moderately susceptible towards the drug and no TCR deficiencies in breast cancer cells have been detected, suggesting that DNA damage produced by the drug is effectively repaired. Hence, the central hypothesis to be addressed in the proposed research is that the low sensitivity of breast cancer cells toward irofulven correlates with highly effective removal of the formed DNA lesions by TCR, and that inhibiting TCR will amplify tumor DNA damage and enhance the rate of apoptosis in breast tumor cells.

The mode of action of the designed agents is proposed to involve trapping TCR proteins in an irreversible complex with DNA. The DNA-drug-protein cross-links are expected to hijack repair and induce cell death. The objectives of this application are to characterize TCR in different breast cancer phenotypes, establish the levels of irofulven and new drug activation in breast cancer cells and develop new tumor-specific DNA alkylating agents to target repair enzymes.

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The proposed work is innovative, because it introduces transcription-coupled nuclear excision repair as a new therapeutic target. The strategy is breast cancer specific by targeting a repair pathway that appears to be highly efficient in breast cancer cells and tumor-specific due to preferential reactivity of irifolven in cancer cells. DNA-TCR cross-linking drugs are expected to be a novel highly effective approach to breast cancer therapy that can potentially contribute to the treatment of various breast cancer types, including BRCA, estrogen-receptor negative, progesterone receptor negative and triple negative tumors, as well as tumors with acquired resistance to conventional chemotherapeutics. Furthermore, mechanistic information and analytical strategies that will be derived from the proposed studies are expected to allow the identification of breast tumors that will specifically benefit from this strategy, as well as further development of a new therapeutics. My long-term objectives are to pursue a professorship at a top-tier research institute and to lead a research group working at the chemistry-biology interface to devise strategies for targeted cancer therapy, with a particular focus on breast cancer and the proposed training plan is necessary step for achieving my goals. It will help me to build a strong informational foundation in the field of breast cancer therapy, develop expertise in research techniques and practical experimental skills, and will help me to gain knowledge how to build an innovative and productive research program.

Pending Execution of Grant Agreements

PI Name: Douglas Yee, PhD

Institution: University of Minnesota at Twin Cities

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: AIB1 (amplified in breast cancer 1) is a key regulator of the IGF pathway in triple negative breast cancer.

Abstract

Breast cancers that lack detectable quantities of estrogen, progesterone and HER2 receptors are clinically classified as triple negative or basal-like cancers. While triple negative breast cancer patients respond favorably to chemotherapy, they generally have a poor prognosis and no targeted therapies have clearly been shown to provide clinical benefit for women with this disease. Therefore, finding effective targeted therapies for triple negative breast cancers is a high priority.

Recent studies and our preliminary studies suggest that triple negatives cancers may have higher than normal IGF (insulin and insulin-like growth factor) activity or dependency. IGF signaling has been shown to be necessary for maintaining breast cancer growth, metastasis, increased metabolism and cell survival. Unlike other prognostic markers, such as HER2, IGF activity is not simply marked by higher IGF receptor quantities or mutations that leave the receptor “on” all the time. Determining alternate markers or other proteins responsible for IGF signaling in triple negative breast cancers need to be identified in order to choose the right patients that will respond to anti-IGF directed therapies.

We hypothesize that the coactivator AIB1 is a key player in controlling IGF dependent growth of triple negative breast cancers. Previous studies have demonstrated an important oncogenic role for AIB1 in IGF-I driven breast cancers, however, a role for AIB1 in triple negative breast cancers has not been examined. Through the use of triple negative breast cancer cell lines and animal models we will provide evidence that AIB1 is necessary for IGF dependent growth of triple negative breast cancer cells. We will control AIB1 levels using RNA interference to specifically reduce AIB1 in triple negative breast cancer cells. We will also correlate AIB1 expression with sensitivity to IGF-I receptor inhibition. We will also test the affect of AIB1 on IGF dependent growth and metastasis in an immune compromised mouse model.

Since AIB1’s main function is to increase the expression of genes (i.e. a transcription factor coactivator) we further hypothesize that AIB1 must regulate IGF dependent genes that control tumor cell growth and metastasis. We will measure AIB1 regulated genes by quantitative real time PCR and measure protein level changes. Finally, we will determine how AIB1 is “turned on” by the IGF pathway by measuring whether AIB1 is phosphorylated in response to IGF treatment. This would provide a specific method by which the IGF pathway can switch on the function of AIB1. Analyzing AIB1 in triple negative breast cancer will help to distill key IGF molecules that can be used to clinically classify tumors that could benefit from anti-IGF directed therapies.

Results from these studies will have an immediate benefit to triple negative breast cancer patients because our pre-clinical findings can be applied in the selection process for patients to receive anti-IGF therapies. Currently more than ten IGF1R targeted therapies are in pre clinical, phase 1 and phase 2 trials. Anti-IGF therapies have already shown tremendous benefit as a single agent therapy in treating other cancers, such as Ewing’s

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Sarcoma. Anti-IGF therapies have the potential to be hugely beneficial to triple negative breast cancer - if we can give them to the right patients.

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PI Name: Keith Knutson, PhD

Institution: Mayo Clinic and Foundation, Rochester

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Combination therapy for breast cancer using anti-PD-1 antibody and “infrastructure” vaccine.

Abstract

Breast cancer is a malignant tumor of the breast. Conventional breast cancer therapy (radiation and chemotherapy) can kill these breast tumor cells by causing DNA damage. Breast tumors can become resistant to these conventional therapies by developing and upregulating DNA repair mechanisms which repair the DNA damage. Our laboratory has identified and characterized a DNA repair pathway, called the Fanconi Anemia/BRCA pathway, which allows breast tumor cells to repair DNA damage. More recently, we have characterized a novel class of drugs which can inhibit the FA/BRCA pathway. We propose that these new drugs (FA/BRCA pathway inhibitors) can be used to block DNA repair in human breast tumors and to enhance the activity of conventional breast cancer therapy. We plan to determine whether these new FA/BRCA pathway inhibitors can enhance the activity of a new class of DNA repair inhibitors, called PARP inhibitors, when these two inhibitory drug classes are used in combination.

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PI Name: Fergus Couch, PhD

Institution: Mayo Clinic and Foundation, Rochester

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Characterization of BRCA2 variants of uncertain significance (VUS) using genetic and functional approaches

Abstract

The breast-ovarian cancer syndrome is characterized by the clustering of breast cancer or breast and ovarian cancer in families. Inherited mutations in the BRCA1 or BRCA2 genes account for over 50% of inherited breast cancer and result in up to an 80% lifetime risk of breast cancer. Most of the deleterious mutations in BRCA1 and BRCA2 that are associated with inheritance of breast cancer are inactivating mutations that produce an incomplete and non-functional protein. However, clinical genetic testing of the BRCA1 and BRCA2 genes for mutations as a method for identifying women at increased risk for this disease has also identified thousands of missense mutations that cause a change of a single amino acid in the BRCA1 or BRCA2 proteins. The effect of these so-called VUS (Variant of Uncertain Significance), on the protein function is unknown and as a result it is also not known whether the VUS are cancer causing or completely benign. Whereas individuals found to carry inactivating mutations in BRCA1 and BRCA2 receive risk assessment and counseling based on a known diagnosis of a deleterious mutation and can benefit from medical and surgical prevention and specific therapeutic measures, individuals with VUS receive no benefit from clinical testing because of the unknown relevance of the VUS to cancer risk. Over 800 unique BRCA1 and 1000 unique BRCA2 VUS have been identified in over 20,000 families in the USA alone. Various approaches have been developed in an attempt to classify VUS. Specifically the scientific community is studying the effects of these VUS by collecting information on how the VUS track with cancer in families which allows calculation of the odds of cancer causality for VUS found in large numbers of families. As this approach is limited by the numbers of families with each mutation, others have developed laboratory based tests that can measure the influence of the VUS on BRCA1 or BRCA2 activity. It is hypothesized that disruption of these functions of BRCA1 and BRCA2 results in increased risk of cancer. Here we propose to study VUS in the BRCA2 gene and to use three functional tests to characterize VUS as either benign or deleterious. For this project we propose to: 1) To establish the sensitivity and specificity of the three laboratory tests assays. This involves determining if the tests can always correctly distinguish between benign and deleterious VUS by comparing the results of the tests with results from the best method for classification, which is the family method outlined above. This will be accomplished using information ENIGMA (Evident-based Network for the Interpretation of Germline Mutant Alleles) a new consortium organized to improve classification of VUS. In addition, we propose to: 2) use the established tests to predict whether many other VUS in BRCA2 are deleterious or neutral. At the conclusion of the study we expect to have establish the usefulness of several tests for classifying BRCA2 VUS and to have subsequently classified many VUS to the point that the information can be used in risk assessment to aid in the selection of appropriate clinical care for carriers of these VUS.

Pending Execution of Grant Agreements

PI Name: James Hsieh, MD, PhD

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

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: The role of *taspase1* in HER2/Neu driven tumorigenesis

Abstract

HER2 positive breast cancers represent approximately 25% of breast cancer cases. It is defined by the gene amplification and over expression of the HER2 protein. HER2 is a member of the epidermal growth factor receptor family, and it directly contributes to breast cancer proliferation. In addition to standard chemotherapy agents, two FDA-approved drugs are currently available for its treatment that specifically targets the HER2 protein. The inception of targeted therapeutics has revolutionized our current oncology practice and dramatically impacted the survival of this subset of breast cancer patients. Over time, resistance to the HER2 specific treatments develops. As a result, the need for development of new drugs for HER2 positive breast cancer is imperative.

Taspase1 (*Tasp1*) is an enzyme that specifically cleaves TFIIA and MLL to regulate cell cycle control. Cells lacking *Tasp1* exhibit disrupted cellular proliferation and are resistant to oncogenesis. These observations implicate the inhibition of *Tasp1* can be developed as a target for cancer therapy.

An animal mouse model has been developed where the over expression of HER in breast tissue results in mammary breast tumors. Our preliminary data demonstrate that mice lacking *Tasp1* in breast tissue do not develop HER2 driven breast cancer. Based on our preliminary findings, we propose that *Tasp1* is needed for HER2 driven breast cancer, and wish to demonstrate that *Tasp1* is needed for HER2 induced tumor maintenance. Finally, we will investigate the mechanism by which *Tasp1* contributes HER2 driven breast tumorigenesis.

In a parallel with this project, development of a putative *Tasp1* inhibitor is in progress. The data from this project will provide the necessary justification for the future application of *Tasp1* inhibitors in HER2 positive breast cancer in a clinical trial.

Pending Execution of Grant Agreements

PI Name: Marilie Gammon, PhD

Institution: University of North Carolina at Chapel Hill

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Identifying modifiable determinants of cardiovascular disease outcomes among breast cancer survivors

Abstract

Survival among women with breast cancer has greatly improved over the past several decades yielding over 2 million breast cancer survivors currently in the United States. Because they are living longer after diagnosis many of these women are dying from conditions unrelated to their cancer diagnosis, such as cardiovascular disease (CVD). CVD is the most common non-cancer cause of death among breast cancer survivors, and among older survivors CVD accounts for 35% of all non-breast cancer related deaths. However, there has been little research on the causes of CVD among breast cancer survivors. This may be especially important since breast cancer and CVD share a number of risk factors such as poor diet, obesity, and physical inactivity, so women with breast cancer are more likely to be at high risk for CVD when they become diagnosed with breast cancer. Additionally, previous studies have shown that most women tend to gain weight and reduce their physical activity levels after being diagnosed with breast cancer, which could subsequently increase their risk of CVD. Identifying what factors are associated with these conditions could help us to design strategies targeted specifically to breast cancer survivors in an effort to improve these women's chances of survival.

The primary hypothesis that will be tested in this study is that among women with breast cancer, death due to CVD is associated with factors that women may be able to modify intentionally to reduce risk of CVD-related mortality. A related, but secondary hypothesis that will be tested is that the factors related to CVD-related death operate more strongly among women with breast cancer compared to a group of women without breast cancer. Another hypothesis is that among breast cancer survivors, there are modifiable factors that a survivor can intentionally modify in an effort to reduce risk of developing post-diagnosis CVD-related conditions, like high blood pressure, and high cholesterol. The final hypothesis is that weight change after diagnosis and physical activity levels after diagnosis—likely contributors to CVD among survivors—are associated with other factors that could be modified to increase activity and avoid weight gain among survivors.

To test these hypotheses we will use data from an existing cohort study of women who were diagnosed with breast cancer in 1996-1997, and a comparable group without breast cancer. The proposed study would collect data from a national database of death records to identify the women who have died, and if so the cause and date of death. Statistical models will be used to examine the three Aims. Cox proportional hazards—a standard statistical technique to examine longitudinal data such as these—will be used for the first aim, which is to identify the factors associated with death due CVD; factors to be examined include CVD-related conditions and factors that have been associated with CVD among the general population, like dietary patterns, weight and physical activity before and after diagnosis, cigarette smoking, aspirin use and hormone replacement therapy. The same statistical

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technique will be used to examine the secondary part of the first aim, which is to explore whether the risk factors for deaths due to CVD identified among the breast cancer survivors are the same as those for a similar group of women but without breast cancer. These same statistical technique will be used for the second aim (to identify the factors associated with developing post-diagnosis CVD and CVD-related conditions among a group of women with breast cancer). Another statistical technique called generalized linear mixed models will be applied to the third aim (to identify factors associated with post-diagnosis weight change and physical inactivity among breast cancer survivors – since these are two factors that are known to increase CVD among the general population and also likely to influence CVD among breast cancer survivors). This is a standard method for examining how certain variables, such as weight or physical activity, change over time.

The proposed study is highly innovative as it will be one of the first to attempt to identify modifiable factors related to the development of CVD in women with breast cancer, and the first to include women diagnosed with post-menopausal breast cancer – which is the most commonly diagnosed type among American women and the group most likely to develop CVD after diagnosis. Although CVD represents a significant cause of morbidity and mortality among breast cancer survivors it is understudied in this group of women. The study findings would have significant translational impact, as they could help clinicians recognize breast cancer survivors at high risk for CVD, and develop interventions that may help them live longer. Therefore, the results from this study have the potential to help breast cancer survivors: (1) to increase their chances of surviving once they are diagnosed with breast cancer; (2) to reduce their chances of developing CVD-related conditions, which would improve the survivorship experience; and (3) to identify strategies to help them increase their physical activity levels and avoid weight gain after diagnosis.

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PI Name: Pinku Mukherjee, PhD

Institution: University of North Carolina at Charlotte

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Delivery of immune-modulating agents directly to the tumor site in combination with a MUC1 vaccine for the treatment of breast cancer.

Abstract

Significant strides have been made in the fight against breast cancer as the use of traditional therapies has improved and new targeted therapies have been developed. However, despite these notable advances, approximately 40% of breast cancer patients continue to fail current therapies and ultimately succumb to their disease. Furthermore, although women with metastatic disease cancer enjoy a good quality of life, metastatic breast cancer remains incurable. Tumor vaccines offer a new type of therapy for cancer patients where the immune cells of the body are manipulated to attack tumor cells. Side effects from tumor vaccine have been shown to be minimal, as the responses should be tumor-specific. Similar to vaccines against infections agents like influenza, a target must be identified that is specific to tumor cells and not present on normal cells in the body. Because tumors arise from mutated cells of the body, this can be difficult, but targets have recently emerged as possibilities for tumor vaccines. One such target is the protein Mucin1, or MUC1. MUC1 is expressed in normal breast tissue; however tumor-associated MUC1 is expressed in a different form, allowing for direct targeting of tumor MUC1 that will not recognize normal MUC1. Additionally, this tumor-associated MUC1 is present on approximately 90% of breast tumors and metastatic lesions, and a recent study found that MUC1 showed great promise as a target for tumor vaccines. Our lab has shown the ability of a MUC1-directed vaccine to reduce primary tumor size and metastatic spread using mouse models of colorectal, pancreatic, and breast carcinogenesis. The MUC1-directed vaccine has been able to induce an immune response against tumor cells expressing MUC1, directly killing these cells. However, we have found that once these immune cells reach the tumor site, they can be shut down by factors secreted by the tumor. This “immunosuppression” is another challenge to tumor vaccine therapy, and in this proposal, we plan to block immunosuppression occurring within the tumor environment while administering the MUC1 tumor vaccine.

We have developed an exciting approach to delivering compounds that inhibit immunosuppression directly to the tumor site, which will increase specificity and therefore reduce side effects of this treatment. Because MUC1 expression is altered on tumor cells, we can generate antibodies that only bind to tumor MUC1 and do not recognize MUC1 on normal cells of the body. It has been shown that treatment with tumor-specific antibodies alone can offer a clinical benefit, as they can activate other arms of the immune system to attack the tumor. Additionally, we have been able to attach two compounds that block immunosuppression to this antibody, which will directly deliver these compounds to cells expressing tumor-associated MUC1. It is our hypothesis that a combination of vaccine treatment with these immunosuppression inhibitors will allow the immune system to develop a robust response against MUC1 and kill MUC1-expressing tumor cells. As a result

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of the high levels of MUC1 expression on both tumors and metastatic lesions, this immune response should lead to a decreased tumor and metastatic burden and increased survival.

We plan to test our hypothesis using a mouse model of breast cancer. PyMT mice are genetically manipulated to express an oncogene in mammary tissue, leading to the spontaneous development of breast tumors. The tumors that develop in PyMT mice mimic human breast carcinogenesis, as they express similar tumor markers and metastasize to the same sites – the bone and the lungs. We plan to breed the PyMT mice with mice that have been manipulated to express the human form of MUC1. The expression of human MUC1 in these mice occurs in a pattern and at levels consistent with that observed in humans. As a result, we will generate mice that develop spontaneous breast tumors that express human MUC1, which allows us to study the effects of the vaccine and antibody treatment directed against human MUC1 using mice.

We are extremely excited to perform these studies, as they are the first using MUC1-specific antibodies to deliver immunosuppression blockers to the tumor site and the implications from these studies could be far-reaching. Currently, the immunosuppressive environment induced by the tumor is one of the biggest hurdles to vaccine treatment for breast cancer, underscoring the importance of these studies. Additionally, since we are targeting human MUC1, these experiments can be easily translated into a clinical setting. Further, because the MUC1 expression is altered on metastatic lesions, this type of therapy could be used to target these sites in patients with metastatic disease. Also, if we are able to successfully vaccinate against MUC1, this will result in the development of immune cells that remember tumor-associated MUC1 and will attack any recurring tumors that express MUC1. We hope that the proposed studies will lead to treatment modalities that will be capable of reducing tumor burden, targeting metastatic lesions, and preventing recurrence in patients with breast cancer.

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PI Name: Xiao-Fan Wang, PhD

Institution: Duke University

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: TGF-beta regulates miR-126/126* expression to promote breast cancer metastasis

Abstract

Cancer is a leading cause of death worldwide. Breast cancer is by far the most frequent cancer of women. Every year, 216,000 women are diagnosed with breast cancer in the United States. Metastases are the major cause of death from cancer including breast cancer. To complete the metastatic process, cancer cells have to invade through the basement membrane, intravasate into the bloodstream, disseminate through the circulation, and extravasate to distal tissues/organs. During this process, cancer cells also have to overcome many types of stresses, such as hemodynamic shearing, loss of proper adhesion, nutrient depletion, hypoxia, and accumulation of wastes that may all induce cell death. Only a small number of cancer cells are able to initiate the formation of micrometastases in secondary sites, and an even smaller subset of those micrometastases can evolve into macroscopic metastases. This is mainly due to the unfavorable and often hostile new tissue/organ microenvironment to the disseminated tumor cells. Therefore, the interaction of disseminated tumor cells and the new microenvironment is the key to understand tumor metastasis.

TGF-beta plays important roles in controlling many types of cellular functions in the regulation of development and homeostasis. During tumorigenesis, TGF-beta is known to inhibit tumor formation in the early stage, whereas acts as a potent promoter for late stages of the process, including metastasis. Recent evidence strongly supports the notion that miRNAs are also intimately involved in the metastatic process, likely by regulating the expression of critical genes that enhance or inhibit metastasis. In this proposal, we intend to test the hypothesis that specific microRNAs (miRNAs) are mediators of TGF-beta to regulate various cellular functions in the context of tumor metastasis. In our recent studies, we identified miR-126/126* down-regulation is associated with breast cancer metastasis and the expression of miR-126/126* is suppressed upon TGF- β induction. One of the predicted targets of miR-126/126* is SDF1 (stromal cell-derived factor 1), a chemokine that is involved in the recruitment of several types of stem/progenitor cells, including mesenchymal stem cells (MSCs), in the context of tumor metastasis. MSCs are able to migrate into the tumor microenvironment where the production of SDF1 is the source of chemoattractant, and then form a paracrine loop with the tumor cells to produce other types of chemokines/cytokines, such as CCL5, to promote tumor cell invasion and metastasis. Therefore, we propose to test our hypothesis, TGF-beta enhances tumor metastasis by changing the tumor microenvironment through a new mechanism, the recruitment of MSCs by elevated SDF1 via suppressing miR-126/126* to form a positive feedback loop to enhance the metastatic activity of cancer cells. Findings from this project are expected to contribute to the identification of better biomarkers and potential therapeutic targets to improve the early detection and treatment for breast cancer metastasis.

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PI Name: Christine Ambrosone, PhD

Institution: Roswell Park Cancer Institute, Buffalo

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Interactive effects of cruciferous vegetable intake and NAD(P)H:quinone oxidoreductase 1 (NQO1) functional polymorphisms on breast cancer prognosis

Abstract

Proposal rationale: There are approximately 2.4 million breast cancer survivors in the US. Most of them have been, or are being treated with chemotherapy or hormone therapy. It is of increasing importance to examine how dietary and genetic factors interactively affect treatment efficacy and breast cancer recurrence and survival. NAD(P)H:quinone oxidoreductase 1 (NQO1) is a multifunctional protein which plays important roles in protection of cells against oxidative stress, detoxification of carcinogen, and stabilization of tumor suppressor p53. Due to the genetic variations of the NQO1 gene, individuals with certain genetic variants have unstable NQO1 protein and low enzyme activity. Recent studies demonstrated that low NQO1 activity strongly predicts treatment resistance and poor survival in breast cancer patients. Thus, breast cancer patients with different genetic background of NQO1 may have varied treatment outcomes even if treated with same chemotherapeutic regimens. Cruciferous vegetables are a rich source of isothiocyanates (ITCs), which potently induce NQO1. Increase of NQO1 protein can be achieved by inducers in breast cancer cells carrying low-NQO1 genetic variants, suggesting cruciferous vegetable intake may be able to boost the NQO1 activity, thereby improve breast cancer prognosis in certain patient populations. However, genetic variations in NQO1 gene may also influence its response to ITC-rich cruciferous vegetables. Therefore, NQO1 genetic variation and cruciferous vegetable intake may interactively affect breast cancer recurrence and survival.

Hypothesis and study design: We hypothesize that breast cancer prognosis indicated by recurrence and survival varies among patients with different functional NQO1 polymorphisms, and that the association is modified by intake of ITC-rich cruciferous vegetable. To test our hypothesis, we propose to systemically examine the NQO1 gene to identify genetic variations with biologic effect on basal activity and transcriptional activation by cruciferous vegetables in immortalized cells derived from breast cancer patients. Then the identified functional genetic variants will be tested in a prospective cohort study of breast cancer patients. Two important questions will be addressed: 1. whether breast cancer patients with these genetic variants have different disease free and overall survival time; 2. whether cruciferous vegetable intake can improve breast cancer prognosis in patients carrying different genetic variants of NQO1.

Innovation and Impact: The study will, for the first time, link genetic variants in the NQO1 promoter to their functional consequence in basal activity and transcriptional activation by dietary inducers. The novelty of the proposed study also lies in the idea that dietary approach may be able to modify breast cancer prognosis in patients carrying different functional genetic variants of NQO1. This information could provide valuable guidance for development of dietary intervention strategies to improve breast cancer prognosis. Given

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the fact that intake of dietary inducers, such as cruciferous vegetables, is relatively easy to adjust, the study finding may have an immediate impact on breast cancer prognosis.

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PI Name: Clifford Hudis, MD

Institution: Memorial Sloan-Kettering Cancer Center

Mechanism: Postdoctoral Fellowship - Clinical Research

Application Title: Identifying oncogenic targets in trastuzumab-refractory HER2-amplified and triple-negative breast cancer: A phase I/II trial of EGFR/HER2 and mTOR inhibition

Abstract

Despite advances in the diagnosis and treatment of breast cancer, advanced-stage disease accounts for more than 40,000 deaths in the United States annually. Women with breast cancer that over-expresses human epidermal growth factor receptor type 2 (HER2) are at a greater risk for disease progression and death. Additionally, for women with triple-negative breast cancer, a subtype of breast cancer which lacks hormone receptor and HER2 expression, early relapses and a predilection for visceral metastases has been observed. Current biological therapies for breast cancer are tailored to hormone and HER2 receptor status.

In HER2-positive breast cancer, the use of trastuzumab, a monoclonal antibody that blocks HER2 signaling, has significant clinical benefit for both early and advanced-stage disease. However resistance to trastuzumab has emerged and is a significant clinical dilemma. For triple-negative breast cancers there is no identified target for biological therapy and efforts to characterize molecular lesions that may serve as new therapeutic targets has been the focus of much research. The goal of this project is to further our understanding of trastuzumab resistance, identify oncogenic targets in triple-negative breast cancer, and test a novel combination of targeted agents in both of these high-risk subtypes.

The PI3K-AKT-mTOR molecular signaling pathway plays a central role in cell growth and division. This pathway is dysregulated in both HER2-positive and triple-negative breast cancers and may contribute to therapeutic resistance. Though inhibitors of this pathway have demonstrated efficacy in laboratory models, we have found feedback signals that diminish the extent of the response. To counter this feedback, combination approaches may be needed. In trastuzumab-refractory HER2-positive disease, the dual EGFR/HER2 kinase inhibitor, neratinib, continues to provide clinical benefit. We hypothesize that the combination of the PI3K pathway inhibitor, temsirolimus, with neratinib will result in significant antitumor activity in patients with trastuzumab-resistant HER2-positive disease. For patients with triple-negative breast cancer, overexpression of epidermal growth factor receptor (EGFR) has recently been identified and may serve as a potential target for therapy. Laboratory models show sensitivity to both PI3K pathway and EGFR inhibition, leading us to hypothesize that the combination of temsirolimus and neratinib will benefit triple-negative breast cancer.

This project will assess the safety and efficacy of the combination of temsirolimus and neratinib in an effort to overcome treatment resistance in patients with high-risk subtypes of

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breast cancer. Individually, temsirolimus and neratinib are well tolerated and do not have overlapping side effects. We anticipate the combination to be well tolerated and have clinical benefit for patients resistant to other therapies. To better understand trastuzumab resistance and identify novel targets for therapy, we will obtain tumor samples of advanced-disease prior to the start of therapy. We will compare these tumor samples to patients' primary tumor to determine biomarkers that are associated with resistance to prior therapies and predict responsiveness to the temsirolimus and neratinib combination.

This study is an effort to translate findings from our work in the laboratory to the clinical setting by using two targeted therapies to achieve synergistic anti-tumor effects. This clinical trial is the first of its kind in any cancer to test combined mTOR and EGFR/HER2 inhibition. The use of the novel combination of temsirolimus and neratinib has the potential to provide clinical benefit to patients who are resistant to other therapies. In addition to a provocative therapeutic strategy, our unique approach to the analysis of mechanisms of resistance is likely to yield important information about molecular determinants of resistance to prior therapies. Modeling of resistance may guide the development of future therapy with a more rationale and individualized approach to the treatment of breast cancer.

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PI Name: Maura Dickler, MD

Institution: Memorial Sloan-Kettering Cancer Center

Mechanism: Postdoctoral Fellowship - Clinical Research

Application Title: Characterizing the impact of cancer therapy on fertility and sexual health in women with breast cancer

Abstract

BACKGROUND

Most women treated for early stage breast cancer will become long-term survivors. Since multiple treatment options are available, it is essential to understand the potential side effects of each therapy in order to inform medical decisions. Two consequences of breast cancer treatment that profoundly affect the lives of patients and survivors are sexual dysfunction and infertility. Both chemotherapy and endocrine therapy affect ovarian function, and therefore negatively impact future reproductive potential in premenopausal women during their childbearing years, and contribute to sexual dysfunction in both pre- and postmenopausal women. Infertility and sexual dysfunction affect quality of life in breast cancer survivors. Many women of reproductive age would prefer to preserve their fertility and ovarian function if possible, and would like to know the likelihood of ovarian failure and the potential for future infertility given a specific breast cancer treatment. However, the effects of chemotherapy and endocrine therapy for early-stage breast cancer on ovarian reserve are poorly described and are not well understood. The risk of chemotherapy-induced infertility appears to vary based on age and treatment regimen, but currently available information is insufficient to determine the likelihood and extent of ovarian damage suffered by an individual woman.

HYPOTHESIS

Premature ovarian failure and sexual dysfunction are common and negatively affect quality of life in women with breast cancer who receive therapy. The impact of cancer treatment can be quantified in these patients by 1) measuring serial serum markers of ovarian reserve in premenopausal women and 2) measuring sexual function over time by validated measures in both pre- and postmenopausal women and 3) measuring serial serum markers of estrogen levels and sexual function over time in patients receiving an intervention.

METHODS

In Aim 1, we will characterize the effects of cancer therapy on markers of ovarian reserve in premenopausal women during treatment of early-stage breast cancer. Information on monthly menstrual cycles and reproductive health will be elicited via diaries and questionnaires. In Aim 2, we will prospectively follow a group of women from their initial breast cancer diagnosis throughout treatment and measure the impact of disease and therapy on sexual function. In Aim 3, we will follow serum estrogen levels in 50 postmenopausal women with breast cancer being treated with adjuvant aromatase inhibitors who will be initiated on intravaginal 25µg 17-Beta estradiol for symptoms associated with sexual dysfunction.

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INNOVATIVE ELEMENTS

In Aim 1 we will develop a novel research methodology, using serum biomarkers instead of menstrual cycle as the primary measure for ovarian reserve since return of menses is a poor surrogate for future fertility. In Aim 2 we will characterize the impact of disease, treatment, and interventions on sexual function in women with breast cancer, which has not been done in the post aromatase inhibitor era. In Aim 3 we will evaluate the efficacy and safety of an intravaginal estrogen treatment in women with breast cancer.

IMPACT

1) We will determine the likelihood of infertility from a given treatment regimen, which will assist clinicians/patients with potential efficacy-safety decisions in the adjuvant treatment setting and help patients make informed choices regarding fertility preservation procedures. 2) Information obtained will aid in counseling patients on the relative morbidity of alternative treatment strategies and help design interventions that target sexual dysfunction. 3) Information obtained from the 17-Beta estradiol intervention study will help appropriately and safely treat women with vaginal dryness and sexual dysfunction.

The overall objective of my research plan is to improve patient care, scientific knowledge and clinician education in the important but understudied areas of fertility and sexual function. The interrelated projects described will allow us to better understand and appreciate the burden of side effects on our patients and ultimately improve patient outcomes.

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PI Name: Charis Eng, MD, PhD

Institution: Cleveland Clinic Foundation

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Androgen receptor regulates PTEN in breast cancer

Abstract

How could male steroid hormones help fight against female breast cancers? This is the question we are answering in our project. Breast cancer is the most common cancer in women in the United States. 1 out of 8 women would have breast cancer in their lifetime and, every year, there are ~40,000 deaths from breast cancer in this country alone. Despite tremendous discoveries during the past decades, there remain huge challenges such as highly accurate molecular-based early diagnosis and tailored treatments.

Breast cancer develops as a direct result of un-controlled cell growth in the breast. As the basic unit of the human body, a cell regulates its growth and multiplies (knowing when to stop) through complicated mechanisms called cell signaling pathways. Malfunction of those pathways will result in accelerated growth of cells, resulting in a cancerous growth. A key player in one of these pathways is PTEN. In 1997, our lab first discovered that germline (in every cell of the body) PTEN alterations (mutations) led to increased risk of developing breast cancer. PTEN acts as a guardian regulating proper cell growth. If PTEN is altered or lost, cells grow out of control, resulting in a cancer formation.

Androgen is a sex steroid hormone naturally existing in both men and women. In men, androgen is critical for testicle and muscle development. Androgen also regulates the normal development of the reproductive tract, bone, kidneys and muscle in women. After menopause, the ovaries stop making androgens and at the same time, the risk for women to have breast cancer becomes tripled. To date, androgens have been given to patients as a second line hormonal therapy for advanced breast cancer. However, this therapy is not widely used at the moment given the lack of understanding why androgens can cause breast cancers to shrink.

During the past one year of research, we found that androgens can kill breast cancer cells, by enhancing PTEN function. We also found this effect is mediated by androgen's partner: androgen receptor (AR). Doctors have seen the disappearance of AR in a good fraction of breast cancers, especially in the most highly aggressive breast cancers. Therefore, we examined whether and how loss of AR put patients at increased genetic risk of breast cancer, and in sporadic (not inherited) breast cancers, leads to the disappearance of PTEN. In the breast cancer cells, as soon as we removed AR, PTEN levels decreased, and androgen can no longer enhance PTEN. Furthermore, we identified, for the first time, an AR response element (ARE) by which androgen/AR enhance PTEN, within the PTEN gene. Finally, in a small preliminary study of women carrying germline PTEN mutations, we found that >80% of those with germline ARE mutations had breast cancer compared to those with germline PTEN mutations outside the ARE (28%).

To provide a cogent rational explanation why androgen is an effective breast cancer drug, here, we propose to further investigate the mechanism of how androgens bring back PTEN through AR. We hypothesize that, at a molecular level, this is a complicated effect, involving not only AR but also its other partners, including GATA2 and p53. At the clinical level, we are going to investigate whether women with abnormal germline ARE in PTEN develop more

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breast cancers compared to age-matched controls in a known cancer registry, as well as whether somatic (in tumor only) ARE alterations lead to worse outcomes.

If we are successful, we will be able to, for the first time, demonstrate a novel manner of how androgens can control breast cancer growth in women through the PTEN signaling pathway. It will provide us a better understanding of the crosstalk between male steroid hormones and the important PTEN cell signaling pathway. Therefore, our project is not only a connection that links basic, laboratory-based breast cancer research to clinical medicine, but also a powerful tool to benefit patients from the earliest diagnosis to surveillance to the latest drug discovery and hormonal manipulation as therapy and prevention.

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PI Name: Michael Ostrowski, MD, PhD

Institution: Ohio State University, College of Medicine

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: A systems biology approach for dissecting microenvironment networks in breast cancer

Abstract

With all the progress that has been made in diagnosis and treatment of breast cancer, the predictive aspect for risks, therapies and outcomes for the disease still remain poor. The dual mystery of metastatic breast cancer and selective resistance to drugs has led to a situation where patients have been deprived of appropriate clinical management. Our overall objective is to comprehensively combine known and well-characterized risk factors such as family history and receptor status, with novel markers that accurately indicate important events, early and late, within the tumor microenvironment. The major goal is to identify changes that occur in the stromal cell compartment that surrounds and supports the mammary epithelial tumor cells. Our hypothesis is that the specific stromal cell types associated with mammary tumors are critical to occurrence, relapse and spread of breast cancer. Using the state-of-the-art technique of Laser Capture Microdissection (LCM), I propose to retrieve specific cell populations from the breast tumor microenvironment, in mice as well as humans, and compare each cell type in terms of their gene expression. Combining basic experiments with powerful bioinformatic tools, the ultimate aim is to elucidate the timeline and range of interactions between different cellular components that favor breast tumorigenesis and their ability to develop therapeutic resistance. The overarching objective of the study is to identify biological markers that are clinically applicable to human breast cancer, and can be customized to each individual person.

The role of the microenvironment in tumor progression is an emerging area of research that clearly has important clinical implications for human cancers. The conversion of normal epithelial cells to metastatic tumor cells is accepted as a multi-stage process that requires progressive genetic alterations within the epithelial tumor cell and has been the focus of intense investigation. However, the stroma surrounding the tumor microenvironment is increasingly appreciated as components of a complex biological network that are critical for tumor progression. This research study aims to provide new information on these stromal cells isolated from mouse and human mammary tumor samples. While this would be interesting in itself, we also plan to correlate these findings to clinical outcomes in breast cancer patients by designing a Breast Cancer Predictive Signature (BCPS), which shall take into account multiple levels of relevant information. By the end of this study, we hope to have a comprehensive and useful Clinical Decision Guide (CDG) that can be applied directly in the clinic to help patients survive through their difficult journey. The primary groups that stand to potentially benefit from our study are those patients who currently fall into categories where there is no clear indication of their risk status, therapeutic options or response to treatment. These critical subgroups deserves more than ever for novel biomarkers that will aid the clinician with a decision flowchart towards better long-term management. The future of breast cancer research is in designing studies with end-points that translate as directly as possible to clinical outcomes. It is also essential that such

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research yield results that bring us closer to the goal of achieving personalized medicine. The ultimate goal is to have a direct impact on effectively improving preventive, diagnostic and clinical outcomes in breast cancer. The implications of this research study go beyond breast cancer, and could emerge as a systems biology approach to a host of other diseases.

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PI Name: Arjun Yodh, PhD

Institution: University of Pennsylvania

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Non-invasive tissue response monitoring during neoadjuvant chemotherapy using Diffuse Optical Tomography and MRI

Abstract

Diffuse optical techniques measure changes in low power light intensities after it interacts with tissues. The changes in light intensity communicate functional information of the tissue non-invasively. During the past half-dozen years, scientists have applied noninvasive diffuse optical tomography (DOT) and spectroscopy (DOS) in clinical settings and have demonstrated that intrinsic breast tumor optical contrasts, such as total hemoglobin and tissue oxygen saturation, are detectable. We have shown that cancer tissues are differentiable from normal and non-cancer (benign) tumors using our 3-dimensional (3-D) DOT in our previous publications. Furthermore, recent research has shown that cancer therapies can induce changes in tumor optical contrast, many of which agree with physiological expectations and with observations of other imaging modalities such as MRI. Based on these results, diffuse optical techniques appear especially attractive for cancer therapy monitoring, an application requiring frequent, portable, non-invasive measurements at low cost.

In this study, based on our preliminary data, we anticipate that DOT will be sensitive to tissue functional changes during chemotherapy (hypothesis) and might eventually become a simple and cost-effective method for monitoring treatment efficacy during the therapy. Results from DOT can help in optimizing chemotherapy treatments for each patient and lead to a complete response of the tumor. There are several studies showing that pathologic response after neoadjuvant chemotherapy is predictive of long term survival thus, once DOT is validated through this clinical study, it can contribute to increasing chances of long term survival of the patients. Since this technique is safe (unlike X-ray or mammography that uses harmful ray) and does not compress the breast forcefully, the patient can be measured as often as needed with relative comfort during the measurement, while quantitative tissue physiological information is obtained.

Specifically in our study, we will compare DOT images to magnetic resonance (MR) data (specific aim1). At UPenn we have a unique opportunity to join an ongoing clinical chemotherapy investigation at Hospital of University of Pennsylvania (HUP). The proposed DOT/MRI chemotherapy monitoring study is of particular interest to us because of the enormous MR-oriented technical strength in the HUP; indeed, our group was the first to perform concurrent DOT/MRI of breast [Ntziachristos, V. et al., Proceedings of the National Academy of Sciences, 2000. 97: p. 2767-2772]. Through collaborations with leading radiologists and an oncologist at UPenn (Drs. Mitchell Schnall, Mark Rogen and Angela DeMichele), we will identify tumors and track their optical and physiological property changes over time during neoadjuvant chemotherapy, including size and vascular-related characteristics and compare the images to MR data obtained on the same time points. We will focus on identifying optical parameters correlated with the final chemotherapy responses. The ability of the optical indices for differentiating responder groups at various time points will be investigated.

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In spite of sensitivity and specificity of Diffuse Optics to breast cancer pathophysiological properties, an improved understanding of the physiological origin of optical contrasts at the microscopic level is important for the interpretation of current DOT results. Once proven, although indirect, DOT will be able to provide quantitative information on the amount of biomarkers and microvessel density in a non-invasive way using optical parameters. We will perform a correlation study between DOT images and histopathological results for breast cancer biomarkers (specific aim 2). The histopathological correlation study will be performed in collaboration with Dr. Michael Feldman, the director of Cancer Center Core Tissue Bank and Pathology Informatics. We will perform a retrospective histopathology analysis of biopsy samples from the patients measured with DOT. First, we will quantify microvessel density, mean size and volume fraction of organelles, and the concentrations of biomarkers such as VEGF, HIF-1, Ki67, and Her-2. Then we will correlate these quantities with tumor contrasts extracted from DOT.

In summary, if DOT is validated with statistical significance through this comparison/correlation studies using MR and histological data, DOT will be a relatively simple and cost-effective method that will contribute to increasing long-term survival cases by optimizing treatment with reliable physiological and pathological data obtained non-invasively.

Pending Execution of Grant Agreements

PI Name: John Lazo, PhD

Institution: University of Pittsburgh

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Identifying chemosensitivity nodes and small molecule inhibitors regulating breast cancer stem cell proliferation and survival

Abstract

Existing breast cancer treatment can shrink tumor size substantially but cancer often returns and spreads to other sites. Recently studies suggest that breast cancer growth and spreading is the product of a small population of breast cancer cells that resemble adult stem cells, called “cancer stem cells”. Scientists have isolated cancer stem cells from human breast tumors and have demonstrated that they have much higher potential to produce tumors in animal models than the non-stem cell population. They also found that a higher fraction of breast cancer stem cells is associated with shorter cancer-free interval and overall survival, and with greater incidence of distant metastasis. Breast cancer stem cells are also shown to be resistance to conventional anti- breast cancer chemotherapy. Current breast cancer therapies, which were developed mostly for their activity to inhibit bulk replicating breast cancer cells, might spare enough cancer stem cells to permit tumor regeneration. Therefore, therapies that selectively targeting cancer stem cell population might offer the promise of more effective breast cancer eradication. One promising strategy is to target genes and pathways controlling the proliferation and survival of cancer stem cells in breast tumors. However, these genes and pathways are still not clear so far.

To identify genes/pathways that are critical for breast cancer stem cell proliferation and survival, we used cancer stem cell-like cells derived from breast cancer cell lines as model. These cancer stem cell-like cells share many common properties of cancer stem cell and can generate tumors in animals with as few as 100 cells. It is our hypothesis that these cells possess the genes and pathways regulating breast cancer stem cell proliferation and survival that are absent/inactive in non-stem cancer cells; and we will be able to preferentially kill breast cancer stem cells by targeting these genes/pathways. We took advantage of the small interfering RNA (siRNA) technique that can selectively “silence” or inhibit a gene of interest in cells and then examine how cells respond to infer the gene’s function. We have screened siRNA molecules targeting 5,520 different genes in human cells and identified the genes when silenced lead to breast cancer stem cell death or inhibited growth. We will also conduct high-throughput compound screening to screen 100,000 compounds to identify small molecule chemical compounds that only inhibit the growth/survival of cancer stem cells but not bulk breast cancer cells. We will carry out experiments to validate our findings by inhibiting the identified genes and testing how the inhibition affects breast cancer stem cell growth and survival. The effects of the identified small molecules on breast cancer stem cell growth and survival will be examined as well.

Identification of key genes and pathways in cancer stem cell proliferation and survival would elucidate the mechanism of cancer stem cell maintenance and thus shed light on breast cancer initiation and growth. The findings of this study will also suggest new therapeutic targets and new drugs for more effective breast cancer therapies specifically targeting

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cancer stem cells. These therapies would help women with breast cancer to completely eradicate their tumor and prevent cancer relapse and metastasis.

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PI Name: William Muller, PhD

Institution: McGill University

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: The role of c-Src in ErbB2-driven mammary tumorigenesis and metastasis

Abstract

Genetic changes in breast cancer cells, including gene amplifications, deletions and mutations, alter the activity of cellular signaling pathways. This altered cell signaling is a crucial property underlying many aspects of tumor cell behavior. An important goal of the study of tumor cell signaling is to design drugs that block the activity of abnormal signaling pathways in tumor cells. This should be a highly efficacious strategy that avoids damaging normal cells and hence eliminates many of the harsh side effects of chemotherapy. Several such “targeted therapies” are in clinical use and many more are under development. Approximately 20-30% of breast cancers have amplification of the gene encoding a cell signaling receptor known as ErbB2/HER2 (human epidermal growth factor receptor 2). These tumors express high levels of ErbB2, correlating with a poor prognosis for these patients. Targeted therapies directed against ErbB2 such as trastuzumab, an antibody, and lapatinib, an inhibitor of the enzyme activity of ErbB2, can extend the lifespan of ErbB2-positive breast cancer patients. However, many ErbB2-positive patients do not respond to these therapies, and resistance inevitably occurs in those patients who do respond. The well-known cell signaling protein c-Src is highly expressed and activated in a large proportion of human breast cancers including ErbB2-positive breast cancers. Like ErbB2, c-Src belongs to a class of enzymes known as tyrosine kinases that transmit cellular signals by transferring phosphate groups to other proteins. c-Src binds directly to ErbB2 and is thought to cooperate with ErbB2 to transmit abnormal cell signals promoting tumor growth, protecting tumor cells from death, and inducing the motility of tumor cells and their invasion of surrounding tissue. Interestingly, some evidence suggests that c-Src activation may be involved in resistance to ErbB2-targeted therapies. Because inhibitors of c-Src are also available this raises the possibility of targeting c-Src, a protein required for signalling by ErbB2, to treat ErbB2-positive patients and increase the efficacy of ErbB2-targeting drugs such as trastuzumab and lapatinib. However, most experiments analyzing c-Src function in breast cancer to date have been done under the artificial conditions of tissue culture. While these studies have provided much valuable information, in vitro conditions do not accurately represent the tissue structure and the presence of many other cell types (collectively referred to as the microenvironment) in vivo. Such studies therefore cannot accurately model the highly complex processes of tumor development and metastasis. The importance of c-Src in breast cancer in vivo therefore remains unclear, and there is little evidence to suggest whether or how c-Src inhibitors will work in vivo, particularly for ErbB2-positive breast cancer.

Mouse models of breast cancer are highly useful tools to study tumor cell signalling, tumorigenesis and metastasis in the context of the whole organism. The mentor for this project, Prof. William Muller, has developed models of ErbB2-driven breast cancer reproducing many aspects of the human disease. Importantly, mammary tumors arising in these models mimic human breast cancers in their dramatically increased expression and

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activation of c-Src. Technology for deleting specific genes in the mammary epithelial cells allows the study of signalling molecules in tumor cells in vivo. In this project, we will use these systems to analyze the role of c-Src in ErbB2-driven mammary tumor biology. We will verify the human relevance of our findings by using human ErbB2-expressing breast cancer cell lines and comparing our data with published data from human patients. Our hypothesis is that the deletion of c-Src in mammary epithelial cells in our models will interfere with the growth and progression of tumors and cause the activation of other signaling pathways in ErbB2-expressing tumor cells that can be targeted to impair their growth, survival and metastasis. We will use cutting-edge technology to identify genes involved in compensating for the loss of c-Src in tumor cells, individually block their function, and determine effects on proliferation, death, and invasion. This information will be very important since these compensating pathways may represent new therapeutic targets that can be combined with c-Src inhibition to treat ErbB2-positive breast cancer. We believe that deletion of c-Src will also impair the metastatic ability of tumor cells in our models. We will use the latest technology to examine metastasis to the lung and bones, two sites of major clinical relevance in breast cancer. These findings will be of great significance to breast cancer patients since metastatic disease accounts for most of the morbidity and mortality associated with breast cancer. Our results will establish the possibility of therapeutically targeting c-Src to interfere with ErbB2-driven metastasis and improve the survival and quality of life of patients. Finally we hypothesize that a chemical inhibitor of c-Src will affect tumor progression in vivo in the ErbB2 model, and treatment with this drug or deletion of c-Src will improve the response of the ErbB2 tumors to lapatinib. These results will provide in vivo support for the idea that c-Src and ErbB2 targeted therapies can be combined to increase the therapeutic benefit for patients with ErbB2-positive breast cancer. We hope that the results of the proposed project will be translated directly to the clinic, leading to trials of new therapeutic regimens to develop more effective treatments for ErbB2-positive patients.

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PI Name: Morag Park, PhD

Institution: McGill University

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Investigation into the Role of Met, a Receptor Tyrosine Kinase, in the Development of Basal-like Breast Cancer

Abstract

Human breast cancer is a highly heterogeneous disease known to be sub-categorised into at least 4 broad types known as luminal A, luminal B, Her-2 positive and basal-like. The subtype referred to as 'basal-like' is currently the most challenging form of breast cancer to treat because it does not express standard therapeutic targets, namely the Her2, Estrogen and Progesterone receptors. A clearer understanding of the molecular biology and the cellular signalling pathways that are important to the development and maintenance of basal-like tumors will allow us to design alternative therapeutic strategies for the treatment of patients with this disease.

Our laboratory recently published a mouse model in which the formation of mammary gland tumors is driven by forced expression of the protein Met. These data showed that mammary gland specific expression of Met, a cell surface receptor, led to the formation of tumors with two distinct types of pathology, 50% that are referred to as 'solid' and 50% that are referred to as 'mixed'. Importantly, detailed analysis of gene expression data revealed that solid and mixed tumors were representative of human luminal and human basal-like breast cancer, respectively. As part of the same study, it was confirmed that human basal-like breast cancers express high levels of Met and that this is correlated with poor patient outcome. Another key feature of human basal-like breast cancer is mutation of the tumor suppressor gene p53 and recent work has shown that p53 may negatively regulate Met in normal cells. In order to understand the relationship between p53 and Met in tumors and in order to combine two clinical features of human basal like breast cancer in our mouse model, we have inter-bred mice expressing Met with mice that have lost mammary gland expression of p53. Our preliminary data show that tumors develop much more rapidly in these mice and that 100% of these tumors have basal-like pathology. In addition, these tumors also demonstrate a phenomenon referred to as 'epithelial-to- mesenchymal transition' (EMT), in which cells adopt an elongated morphology and become more migratory.

Hypotheses: 1) We predict that the basal-like tumors formed in the Met/p53 model represent a recently identified human subclass of basal-like breast cancer, referred to as 'claudin-low', in which tumors show increased expression of cancer stem cell markers in addition to markers of EMT. In order to test this, we will draw detailed comparisons of our model with human basal-like breast cancer, primarily at the level of gene expression. We will also study 'micro-RNAs', small RNA molecules that negatively regulate gene expression and that are now known to have a role in the pathology of human breast cancer. Finally, we will look for patterns of chromosomal gain and loss in our mouse model tumors. 2) It is predicted that loss of p53 leads to the expansion of a cancer stem cell population and this could account for the increased formation of basal-like tumors in our model. To address this, we will isolate cells from these tumors and employ well-established assays that assess stem cell behaviour. Most importantly, it will be possible to manipulate gene expression in these

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cells before re-injecting them into the mouse mammary gland, allowing us to determine effects on tumor growth and metastasis.

Mouse models that mimic human breast cancer are extremely valuable because they allow us to manipulate key clinical features of the disease and to observe the consequences of these manipulations directly. Notably however, there are remarkably few mouse models that represent the basal-like subclass of human breast cancer. Specifically, only models involving loss of BRCA1 produce mammary tumors with basal features, but given that not all human basal breast cancers are caused by BRCA1 deficiency, this model is limited in its clinical relevance. By contrast, we have previously estimated that 65% of human basal-like breast cancers express high levels of Met. Furthermore, the vast majority (82%) of basal-like tumors express mutated p53. The observation that mouse mammary tumors driven by expression of Met and loss of p53 display very important features of human basal-like breast cancer, presents a unique opportunity to learn more about this disease. The ability to study basal-like breast cancer in vivo will aid us in the identification of new candidate molecules that could be therapeutically targeted. Furthermore, it will be possible to immediately test the therapeutic potential of these targets. It also possible, through future collaboration with pharmaceutical companies, that our mouse model could be used in pre-clinical trials of new drugs for the treatment of basal-like breast cancer.

Given the currently limited treatment options for patients with basal-like breast cancer, this project has the potential to make significant contributions to the clinical community and to greatly benefit patients.

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PI Name: Michael White, PhD

Institution: University of Texas Southwestern Medical Center

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Genotype-specific microRNA function in breast cancer

Abstract

In a very real way, each breast cancer patient actually has a unique disease. This is because changes in a breast cell's DNA, the underlying cause of breast cancer, occur randomly over time. By the time cancer is detected, many DNA changes have already occurred, allowing the breast cancer cells to grow uncontrollably. However, there are many different ways that DNA can be altered to allow uncontrolled growth, and because the DNA changes occur randomly, the cancer cells in each patient have arrived at this state of uncontrolled growth through a different path. This has implications for disease progression and treatment, explaining why breast cancer is more aggressive in some patients than others and why particular treatments are successful in some patients but unsuccessful in others.

My long-term goal is to establish an innovative breast cancer research program focused on discovering how specific DNA changes contribute to breast cancer progression, including how the unique combination of changes present in a patient's cancer cells determine how that patient will respond to treatment. This research will identify novel targets for personalized or "patient-tailored" therapy, so that eventually clinicians will be able to treat each breast cancer patient with drugs designed to combat that patient's unique disease. The research project and training plan outlined below will enable me to accomplish this goal by providing a wealth of new information about the important similarities and differences between breast cancer cells from different patients, as well as teaching me the skills necessary for a successful career in breast cancer research. Under the mentorship of Dr. Michael White and in collaboration with Dr. Adi Gazdar, I will explore the functions of microRNAs in breast cancer cell lines from multiple patients. MicroRNAs are a newly-discovered type of molecule present in all cells that influence how the cell behaves by controlling many other molecules within the cell. It has already been discovered that breast cancer cells contain different microRNAs than normal breast cells, and even that different sets of microRNAs are present in different subgroups of breast cancers. However, it is not known whether gain or loss of microRNAs actually contributes to breast cancer formation or to characteristics that distinguish one patient's cancer from another's. We predict that certain microRNAs can inhibit breast cancer formation in the context of specific combinations of DNA changes, and so those particular microRNAs are lost during progression of a patient's disease depending on the other DNA changes that have occurred. To test this prediction, we will use tools that mimic every human microRNA one-by-one. These will be introduced into eleven breast cancer cell lines derived from different patients, for which all of the DNA changes are already known. We will determine which microRNA mimics can kill which breast cancer cell lines, thus obtaining a "microRNA sensitivity profile" for each line. Then we will determine which DNA changes control whether certain microRNAs kill the breast cancer cells. Finally, we will sort out how the microRNAs kill the cancer cells by identifying the important molecules being controlled by the microRNAs in these breast cancer cells.

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In the end, both the microRNAs and the molecules being controlled by them will provide important clues for how to treat breast cancer with patient-tailored therapy. Although knowledge of microRNAs is still relatively new, clinical trials are already underway testing drugs that mimic or inhibit microRNAs for treatment of other diseases. This suggests that drug development may be able to move much more quickly to target microRNAs than other molecules already known to be important in breast cancer. On the other hand, the microRNAs identified in this study may control molecules for which drugs already exist, in which case this research would immediately provide information about which patients may be more or less likely to respond to such drugs. In either scenario, the project proposed here will provide insight into the variability of breast cancer from patient to patient, which can be used to develop new patient-tailored therapies that will especially benefit groups of patients for which current therapies are not successful. In addition, by completing this project I will produce data and receive training necessary to begin an independent career as an innovative breast cancer researcher.

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PI Name: Jamboor Vishwanatha, PhD

Institution: University of North Texas Health Science Center

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Development of annexin A2-targeted curcumin-loaded multifunctional PLGA nanoparticles for breast cancer therapy

Abstract

Breast cancer remains an alarming health problem within the United States, which affects more than 200,000 American women per year. It is the most common cancer diagnosed in American women and is second only to lung cancer as the cause of cancer related deaths. These figures point to the urgent need for an advancement in the treatment of breast cancer.

Treatment options for patients with breast cancer were traditionally based on cytotoxic chemotherapy but now include therapies directed towards identifiable targets associated with tumor proliferation and progression. Recent advances in nanotechnology have provided a means by which various biologicals can be delivered to cancer cells for innovative treatment modalities. Often these targeted therapies are efficacious and at the same time less toxic than traditional regimens.

We will utilize a biodegradable, nontoxic, sustained release drug delivery technology targeted to breast cancer. This is essential because we need to have a targeted entity to kill the cancer cells. In order to specifically target breast cancer, we will make use of a specific cell surface antigen known as annexin A2. Annexin A2 is over-expressed by tumor cells in most breast cancers and hence serves as a biomarker and can be used for specifically targeting the cancerous cells.

In our research, we will use curcumin as a drug to treat breast cancer. Curcumin is a natural herbal compound with anticancer properties and has been used as a food supplement over many years. We will prepare curcumin loaded nanoparticles attached to antibodies that would target annexin A2 on the cancer cells and kill them. This results in reducing the dose necessary as compared to free curcumin alone, thereby reducing the side effects associated with high dose of cancer drugs. The use of targeted therapy as well as combination therapy will enhance the therapeutic benefits in breast cancer patients.

Upon successful completion of our studies, we believe that the knowledge we will gather about this novel strategy of nanoparticle based targeted drug delivery will increase our chances of treating metastasized breast cancer and serve as an adjuvant therapy in the treatment of breast cancer.

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PI Name: Catherine Denicourt, PhD

Institution: University of Texas Health Science Center at Houston

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: The role of p27kip1 deregulation during cell migration and metastasis

Abstract

Metastasis is the most devastating aspect of malignant disease, and the major cause of treatment failures for patients diagnosed with cancer. Metastasis is the process by which tumor cells spread from the primary organ in which they arose to other sites in the body. In most cases, tumors that stay confined to one organ will not be fatal, and eradication of these tumors by surgery or radiation often results in complete cure. Tumors that have spread to other organs by the time a patient is first diagnosed with cancer have a substantially greater life threatening potential. To invade into local tissue at the primary site, tumor cells must first migrate away from the primary sphere of tumor cells and then survive in the circulation and the invaded tissue. Thus, increased cell migration is necessary for cells to become metastatic. p27kip1 is a protein that negatively regulates cell proliferation. This protein resides in the nucleus of cells from normal healthy tissue where it acts to inhibit cell division. However in 40% of breast cancer tumor cells it is instead localized in the cytoplasm and this correlates with poor patient prognosis. We have demonstrated that in the cytoplasm, p27kip1 can directly increase cell migration and metastasis. Moreover, cancer cells with cytoplasmic p27kip1 are more resistant to chemotherapeutic agents. We hypothesize that cytoplasmic p27kip1 plays a very important role in breast cancer progression and metastasis. Using biochemical approaches to elucidate the mechanism by which p27kip1 regulates breast cancer metastasis, we identified two novel cytoplasmic p27kip1-interacting proteins that are associated with cell migration. We will define whether these interactions are necessary for cell migration and metastasis through the use of cell-based assays and mouse models. Ultimately, the findings proposed in this study will help establish approaches to block cytoplasmic p27kip1 oncogenic functions and thus have a major impact on the treatment of breast cancer metastasis. Moreover, understanding how p27kip1 influences metastasis will further help in the design of new strategies to cure breast cancer.

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PI Name: Prahlad Ram, PhD

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

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Targeting acquired Lapatinib-resistance in breast cancer

Abstract

Breast cancers have recurrent overexpressions in the ERBB2 gene, which codes for a protein involved in a major signal transduction pathway involved in cell growth and survival of cancer cells. Therefore, targeting this protein in the therapy of these breast cancers is an attractive therapeutic strategy, which has been employed by some recent drugs, trastuzumab and lapatinib. Lapatinib is a specific small molecule inhibitor of the ERBB2 protein, which can cause death of cells overexpressing this receptor, and it is being widely used in clinic for the treatment of ERBB2-positive breast cancers. However, a large percentage of breast cancers do not respond to this drug despite having overexpressions in the receptor, and still many gain resistance to this drug and result in tumor relapse. In order to understand the molecular mechanisms of this resistance, we developed a cell culture model of lapatinib resistance. We continuously passaged a lapatinib-sensitive ERBB2-positive cell line (SkBR3) in increasing doses of lapatinib till they developed an almost 100-fold resistance to this drug. Then we performed a genome-scale gene expression measurements in these two cell lines to find which gene programs are different and therefore more likely to play roles in the resistant vs. sensitive cells. We identified several molecular processes that are likely to play important roles in lapatinib-induced cell death in the sensitive cells, and possible mechanisms of evasion of cell death by the resistant cells. Specifically, we find large changes in genes coding for specific metabolic pathways involved in energy production, reactive oxygen species generation and the oxidative consumption of glucose for the generation of building blocks for the cell. The gene expression data suggest that lapatinib-resistant cells are utilizing an alternative strategy of energy production and generation of building blocks that may allow them to survive harsh intracellular conditions after the inhibition of EGFR/ERBB2 signaling. We propose several sets of experiments to test these hypotheses and to potentially reverse the resistance of lapatinib-resistant cells. The gene expression data also suggested that a major signaling pathway involved in the inhibition of cell growth, namely TGFbeta pathway, is significantly down-regulated in the resistant cells relative to sensitive cells. This may contribute to the increased survival of the lapatinib-resistant cells. The experiments proposed here also address the hypothesis that the TGFbeta pathway may be involved in lapatinib-induced cell death of breast cancer cells, and that lapatinib resistance may be associated with a specific shut-down of this pathway. Overall, this study addresses important problems in breast cancer therapy, and has the potential to unveil novel mechanisms of resistance of breast cancer cells to targeted EGFR/HER2 therapy.

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PI Name: Gordon Mills, PhD

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

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Role of Rab25 in progression of breast cancer

Abstract

In normal cells, growth factors and nutrients in the extra-cellular environment provide vital cues for cell growth, division or even movement, such as during wound healing. Normal cells have an intricate and highly regulated system of importing growth promoting cargos from outside to inside, using protein sensors, called receptors, which reside on cell membrane. Once these receptors, which are partially extended outside the cell, bind to their cargo, the total complex is internalized by the cell via a process called endocytosis. The 'growth-factor activated' receptor now relays signals to a cascade of proteins such PI3K/AKT pathway or RAS/MAPK pathway, both of which promote cell survival, replication, motility and prevents programmed cell death. Simultaneously, the receptor and its cargo are channeled through several sub-cellular compartments, each marked by specific 'Rab-family' proteins. The purpose of this trafficking is to sort activated receptors into two broad categories: some receptors are recycled back to the membrane to continue signaling, while majority are send to an acidic compartment for degradation, thus attenuating the signal. Somehow, in cancer cells this endocytic recycling regulation is lost. Growth-factor-bound activated receptors are constantly recycled, seldom degraded, promoting a constant flux of growth signals to the cancer cell.

Analyzing a large body data from breast cancer patients, we identified a protein named Rab 25, which was abnormally active in the majority of hormone receptor positive (HR+ve) breast cancers and strongly correlated with their poor outcome. Residing in distinct cellular compartments, the Rab proteins function as molecular switches which can be turned on and off. For examples Rab11A and Rab 25 lead to recycling of cargo, where as Rab7 or Rab 9, channels cargo for degradation. Interestingly, the biochemical structure of Rab 25 suggests that this protein is unique and cannot be turned off. In normal cells this problem is overcome by maintaining very low levels of Rab 25 and high levels of its competitor, Rab11A, which fortunately can be turned off.

Objectives

An exact opposite effect was observed by us in HR+ve breast cancers, with increased levels of Rab 25 and low levels of Rab11A. Functionally, Rab25 and Rab11A typically interact with a third protein called RCP (Rab Coupling Protein), which when activated, recruits receptors and directs them towards recycling. So potentially, for excessive recycling to occur, as reported in many types cancers, two things could happen :1) active Rab 25 could generate active RCP once it replaces the competitor Rab11A from RCP; or 2) if RCP levels were much increased than Rab11A levels, the cell would fail to balance between receptor recycling and degradation. Indeed, when we looked at breast tumors, not only were Rab 25 levels increased but RCP levels were also coordinately increased in multiple clinical data sets. Based on these observation we hypothesize that: Active Rab25:RCP complex formation in HR+ve breast cancer increases recycling of growth factor receptor and nutrients, thus enhancing survival and proliferation signals, leading to a worsened outcome for patients.

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To test this, first we will genetically manipulate Rab 25, Rab11A and RCP to change their activation and binding status. We will use breast cancer cell lines which are sensitive to growth factor stimulation. Next, we will test how these alterations affect individual steps of endocytosis like recycling vs. degradation. We will fuse fluorescent red and green colored proteins with our target proteins to easily detect their cellular localization under the microscope. Furthermore, we will measure cell proliferation, cell death and cell motility following each of the genetic manipulation to support that only activated Rab25:RCP complex can promote recycling of receptors and nutrients to drive excessive cell growth and movement. We will also measure the levels of activated AKT and MAPK as a test for increased survival signaling in these cells. Then using high through-put genomic and proteomic screens, one of the biggest strengths of our laboratory, we will identify sets of genes and protein that were altered due to coordinated activation Rab 25:RCP.

Significance

HR+ve tumors are the most frequent subtype of breast cancer. Although initially patients respond well to Tamoxifen treatment, but these tumors recur after prolonged periods of time. Overall more women die from HR+ve breast cancers than from any other group and finding an alternative treatment for these patients has been a long unmet need. The fact that patients with increased levels of both Rab25 and RCP show a worsened outcome than those with increases in only one protein supports a critical role for Rab25: RCP interactions in breast cancer progression. Till date most of the Rab protein interactions are limited to genomic studies and our work will use the great opportunity to understand its implication in breast cancer. Our novel approach to target the cell's own recycling system becomes even more important in breast cancer because growth factor receptors are often over-expressed in breast tumors. Drugs like Gefitinib, which target receptors, loses its potency after a period of time possibly because of this derailed receptor-recycling process. Thus Rab25 strongly emerges not only as a marker for HR+ve breast cancer but as a potential therapeutic target. Based on our observations, GlaxoSmithKline has developed a program to develop Rab25 inhibitors. Their encouraging initial results will allow us to translate our observations to the patient soon.

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PI Name: Mauro Ferrari, PhD

Institution: University of Texas Health Science Center at Houston

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Nested nanoparticles for chemotherapeutic synergy enhancement in breast cancer

Abstract

The long-term goal of the proposed work is to effectively target a specific, molecular pathway that is dysregulated in breast tumors, all with the hopes of generating a more efficacious treatment strategy. Currently, a rising trend in cancer chemotherapy is the design and discovery of anticancer drugs that exert their effects on molecular components found to be either overexpressed in tumors or essential for tumor survival and propagation. For example, the drug rapamycin was shown to yield limited regression of breast tumors by targeting the PI3K/Akt/mTOR signaling pathway, a pathway that is abnormal in a large population of breast tumors. However, it was found that the antitumor efficacy of rapamycin can be greatly enhanced if the anticancer drug paclitaxel is administered 12-24 hours prior to rapamycin. In fact, present-day chemotherapy in breast cancer consists of the administration of several different drugs, some specific and others more all encompassing with regards to their cell killing potential. And while drug synergy is routinely sought after in the clinics, a drawback is that drugs suffer from different distributions in the body, negating any synergistic or additive effects at the tumor site, and oftentimes still resulting in patient toxicity. By delivering the drugs in a more site-directed manner, allowing for enhanced accumulation and controlled release of drugs within the tumor, maximum antitumor treatment can be achieved. Currently, drug-containing spherical nanoparticles with dimensions ranging from 10-1000 nm are enabling the clinical use of drugs, offering advantages such as increased solubility, protection against degradation, and site-specific delivery. Therefore, the purpose of this study is to encapsulate the drugs rapamycin and paclitaxel within a nanoscale particle. The drug carrier will be designed in such a way that rapamycin is encapsulated within the core of the nanoparticle, while paclitaxel is contained in an outer shell surrounding the particle so as to achieve the delivery of paclitaxel first, followed by rapamycin 12-24 hours later. We hypothesize that this "Russian nested doll" delivery approach, in which rapamycin and paclitaxel are delivered within the tumor in a site-, sequence-, and time-specific manner, will provide for an effective nanotherapeutic platform for the treatment of breast cancer. The specific aims of the proposed work are the following: Aim 1) Fabrication and characterization of nested nanoparticles containing a rapamycin-loaded core and paclitaxel-releasing shell; and Aim 2) In vitro and in vivo efficacy examination of the nested nanoparticle platform in breast cancer cells.

Successful completion of the aims highlighted above stand to uniquely lead to a marked reduction in breast cancer mortality. Firstly, we are proposing a novel nanotherapeutic strategy that will ensure proper delivery of drugs to the tumor tissue, significantly reducing toxic side effects and morbidity associated with traditional chemotherapy. Secondly, we are specifically utilizing a chemotherapeutic regimen, consisting of paclitaxel combined with rapamycin, which targets a specific pathway found to be aberrant in a large population of breast cancers. Effective delivery of these drugs to the appropriate cells should target this pathway and lead to greater tumor cell death given the

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highly specific mechanism of action. Last but certainly not least, the novel nanotherapeutic platform that we have proposed is innovative in that few drug nanocarriers exist that are capable of delivering drugs in a time- and sequence-dependent fashion. Hence, this opens up several avenues for the use of this platform with other chemotherapy strategies in breast cancer. It is the hope that the project will help inspire the continued exploration and discovery of anticancer drugs that act on specific molecular markers, as well as provide an advocating voice for exploring synergy among different drugs as a way of treating breast cancer. Hopefully this platform will allow for clinical translation of the synergy observed between different drugs, and more personalized chemotherapy.

The proposed work has widespread and significant implications for breast cancer patients. It is now well-known that chemotherapy is an important adjuvant therapy used in the treatment of breast cancer. However, there is significant toxicity associated with the strategy due to the nonspecific distribution of drugs to healthy organs and tissues, which also contributes to a lack in efficacy and an increase in patient mortality. The nanotherapeutic strategy that we have proposed herein will guarantee delivery of an efficacious combination of drugs to the tumor site, where the drugs will be released in an order and time interval that has been shown to be effective against breast tumors. The PI3K/Akt/mTOR signaling pathway has been shown to be abnormal in a large population of breast tumors. Hence, specifically targeting this pathway with these nanoparticles should bring about increased patient compliance, higher tumor regression rates, and increased breast cancer patient survival rates.

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PI Name: Wendy Woodward, MD, PhD

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

Mechanism: Post Doctoral Fellowship - Translational Research

Application Title: Targeting microRNAs to alter radiation resistance of breast cancer stem cells

Abstract

Cancer stem cells are a small fraction of cells within a tumor that drive the formation, spread, and recurrence of malignant tumors. There is mounting evidence to support the conclusion that cancer stem cells are resistant to radiation treatment, an important component of breast cancer therapy in early and advanced disease. However, the details underlying why and how they are resistant have not been fully explored, and this is important for designing effective therapies to overcome resistance of these critical cells and better treat and prevent recurrence of cancer. Our laboratory has identified differences in specific microRNA expression between cancer stem cells vs. non-cancer stem cells following radiation treatment. MicroRNAs are tiny RNA molecules that are powerful natural repressors of gene expression and may represent a class of regulators that could be important in revealing these mechanisms. Importantly, these molecules can regulate many cell signaling pathways simultaneously and may be more effective anti-cancer targets because they don't leave "back-door" pathways unblocked for cancer to find a way to survive the way targeting a single signaling pathway may. Based on our preliminary data, we propose the central hypothesis: MicroRNAs can be altered to sensitize cancer stem cells to radiation therapy. I intend to 1. Examine miRNAs differentially expressed in breast cancer stem cells versus non-cancer stem cells following radiation treatment. 2. Validate the functions of selected miRNAs (from Aim 1) in altering radiosensitivity of cancer stem cells using miRNA inhibitors/mimics. 3. Study the mechanism of radiosensitization through target protein and pathway identification

From the proposed investigations we expect to identify microRNAs that could be selectively targeted to sensitize breast cancer stem cells to radiation while sparing normal tissue. This work is significant because clinically, radiation therapy is less effective in patients with basal-type, estrogen receptor negative tumors with stem cell features that dominate the clinical picture for many young women and African-American women with breast cancer as well as women with inflammatory breast cancer. Selection and/or development of tumor stem cell radiosensitizers is expected to substantially reduce breast cancer recurrence and improve breast cancer survival especially for breast cancer patients who currently have poor outcomes and limited therapies.

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PI Name: Pothana Saikumar, PhD

Institution: University of Texas Health Science Center at San Antonio

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: Targeting LC3-SQSTM1/p62 signaling axis to treat apoptosis-resistant and metastatic breast cancers

Abstract

Despite significant progress in breast cancer treatment, about 40,000 women identified with the disease still die every year. About ~18% of breast cancers are triple negative (negative for expression of estrogen and progesterone receptors (ER/PR) and HER2 protein) that afflict minorities and younger women. Approximately 50% of breast cancers that are sensitive to hormone, radiation and chemotherapy induced apoptosis during their early stages become resistant as they progress toward more invasive and metastatic property. Therefore, it is important to find suitable and effective targets for the treatment of resistant and triple negative breast tumors.

Rationale, Hypothesis and Objective: Proliferation of normal cells requires the availability of mitogenic factors and substrata for attachment. However, tumor cells show independence from exogenous growth stimuli and attachment factors, acquire self-sufficiency in growth signals and proliferate rapidly. Rapid proliferation of cancer cells requires accelerated rates of protein synthesis. Newly synthesized proteins require maturation by proper folding of peptide chains involving correct interactions between pairs of sulfhydryl groups on peptides. According to my hypothesis, drugs that interfere with protein folding will lead to the formation of misfolded protein aggregates which may perturb cancer cell growth. My preliminary data leads me to believe that rapidly dividing cancer cells will be sensitive to drugs that cause protein misfolding and aggregation by interfering with protein sulfhydryls, thereby inducing non-apoptotic, non-autophagic cell death. I have identified two prototype drugs that exhibit these properties: 15-deoxy-delta^{12,14}-prostaglandin J₂ (15d-PGJ₂) and Manumycin A (Man A). Moreover, I have found that the novel death pathway triggered by these agents involves signaling by two gene products, microtubule-associated protein 1 light chain 3 (LC3) and sequestosome1 (SQSTM1/p62). My proposal is focused on the investigation of how these proteins interact with the cellular machinery that disposes improperly folded and aggregated proteins and on testing the principle using mouse models that the prototype drugs that I have identified have promise in breast cancer treatment. How the present study will advance our understanding of breast cancer and lead to reduction of breast cancer progression and mortality: Deregulation of the death pathway involving programmed cell death (apoptosis) has been implicated in breast cancer progression. Cancer cells may escape apoptosis by either inactivating pro-apoptotic genes or increasing the expression of anti-apoptotic factors. Because resistance to conventional treatment involves the avoidance of apoptosis, it has become important to develop an alternative way to induce cell death in resistant breast cancers. My proposal involves the foundation of a scientific basis for developing sulfhydryl reactive drugs to treat apoptosis resistant breast cancers by inducing an alternative death pathway. Successful use of such drugs to reduce breast tumor burden and inhibit metastasis in animal models will pave the way for further development of promising chemical structures to identify candidates that could reach clinical trials. My study is innovative in terms of basic science as well as

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translational science in developing novel therapies for apoptosis resistant breast cancer. The approach that we are proposing could also reduce the recurrence of tumor caused by mutations attributable to chemotherapy and radiation. An essential feature of the proposed treatment is that it will not affect the genomic stability of normal cells, thus avoiding the causation of second primary tumors following treatment. Furthermore, should mutations arise in cancer cells treated in this manner, the transformed cells that arise by such mutations will fail to grow because of the cytotoxic effects of protein inactivation and aggregation.

The Importance of the proposed Research to patients with Breast Cancers:

Triple negative breast cancer (TNBC) is an “important area of research” of the Susan G. Komen Foundation because it represents a significant percentage of all breast cancer patients (~20%). These patients have poor prognosis with high rates of relapse and short survival, and no targeted therapy has been found. Thus, new agents or combinations of agents are needed in TNBC to prolong survival and improve the quality of life of these cancer patients. After completing this project it is our expectation that we will have not only provided a rational basis for the use of sulfhydryl reactive compounds such as 15d-PGJ2 and Manumycin A to interfere with proteins in rapidly growing cancer cells but also characterized in detail the death pathway involving novel signaling molecules (MAP1-LC3, sequestosome1/p62) that may lend themselves to the development of new classes of drugs through chemical biology screens. The overall purpose of the proposed study is to reduce breast tumor burden, decrease metastasis and improve the quality of life of breast cancer patients including the high proportion of women from minorities who suffer from treatment resistant and triple negative breast cancers.

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PI Name: Nancy Hynes, PhD

Institution: Friedrich Miescher Institute for Biomedical Research

Mechanism: Post Doctoral Fellowship - Basic Research

Application Title: The role of Ret receptor in breast cancer: implications of Ret activation in endocrine resistance

Abstract

Breast cancer is one of the most commonly diagnosed human tumors, affecting one in eight women, and it is a leading cause of cancer-associated death worldwide. After a quarter century of rapid advances, cancer research has generated a rich and complex body of knowledge, revealing that cancer is a disease involving changes in the genome. Normal cells require growth signals in order to move from a quiescent state into an active proliferative state. These signals are transmitted into the cell by transmembrane or intracellular receptors that bind distinctive classes of signaling molecules: diffusible growth factors, hormones, extracellular matrix components, and cell-cell adhesion molecules. Members of the receptor tyrosine kinase (RTK) family represent a major class of proteins that are involved both in growth and differentiation of normal cells, as well as cancer formation and metastasis. As a result, RTKs are being actively studied as targets for therapeutic intervention. Several RTKs have been found to be abnormally expressed in breast cancer, including Ret, which was recently described and is the major topic of this study. In the clinic, endocrine therapy with the anti-estrogen tamoxifen has been of major benefit for treating estrogen receptor positive (ER+) breast cancer. But unfortunately, one-third of women treated with tamoxifen for 5 years will have recurrent disease within 15 years, and so endocrine-resistant disease may represent up to one-quarter of all breast cancers. Insights into the mechanisms of resistance have suggested possible therapeutic approaches for treating endocrine-resistance ER+ breast cancer including the use of tyrosine kinase inhibitors. Based on preliminary data, we hypothesize that Ret might contribute to escape from hormonal therapy. Our goal is to develop systems to test this hypothesis.

The major aims to be undertaken during this project are examine the interaction between the ER and the Ret signaling pathways using cell culture models of ER+ breast cancer cells in order to uncover endocrine resistance mechanisms; and determine the role of Ret in mammary tumor outgrowth of ER+, Ret receptor positive (Ret+) mammary cancer cell lines in mice. Herein, we will use treatment with Ret specific tyrosine kinase inhibitors to test the role of Ret activation in the metastatic process and in endocrine resistance.

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PI Name: Evelinn Borrayo, PhD

Institution: University of Colorado at Denver

Mechanism: Post-Baccalaureate Training in Disparities Research

Application Title: AYUDA: a training program to improve breast cancer outcomes among latina patients

Abstract

TRAINING GOALS AND PROGRAM: The goal of the AYUDA program is to train 3 bilingual doctoral students, preferably of Hispanic (Latino/a) background. They will be trained to create and test assistance interventions for Spanish-speaking Latinas with BC. Such programs could provide patients psychological counseling, help them navigate the medical system, or adjust to issues related to breast cancer survivorship. AYUDA is a term in Spanish that means “help” in English and it will be used as an acronym for program recognition. The long-term goal is to increase the limited number of Latino/a PhD-trained Psychologists. [1, 2, 3] These professionals will have the critical research and clinical skills to effectively address breast cancer disparities by increasing Latina patients’ adherence to their treatment and decreasing high breast cancer mortality.

Three graduate students admitted to the PhD program in Clinical Health Psychology in the department of psychology at the University of Colorado Denver (UCD) will be trained for two years. Students will be trained to develop and test interventions that deal with psycho-social distress and improve adherence of Latina breast cancer patients to their treatment. There will be three training parts: (1) Didactic instruction, (2) Applied Clinical experience, and (3) Applied Research experience. The didactic goals for all students will be to complete: two research method courses, two clinical courses, two health psychology content courses, two public health courses, and two courses on diversity and health disparities. For the “clinical experience,” trainees will practice their skills at UCD one year and another year at the Denver Health Medical Center (Denver Health), which is the hospital for the underserved in Denver, Colorado, with a 60% Latino/a patients. The “research experience” will consist of trainees’ experience designing, developing, and testing behavioral interventions. Such experience will be acquired by conducting two types of research: (a) formative research and (b) pilot testing/evaluation of the interventions.

RESEARCH PROJECT: (2) Study Hypotheses and How it will be Tested – The research aspect of the training is exploratory and thus will be guided by research questions instead of hypotheses. Formative research questions will be: (a) what are the psychological and physical challenges that Latinas face that appear to impact their treatment adherence? (b) what treatment decisions are most difficult to make and then follow through? (c) what are the cultural, educational, and language barriers that interfere with Latinas’ treatment adherence and completion? The pilot/evaluation research questions will be: (a) what aspects of the intervention are well received or need to be modified? (b) what aspects are feasible to implement and at what point during the treatment continuum? (3) What are the advantages and disadvantages of each behavioral intervention protocol and which is most cost-effective? We expect that the findings will point to various psychosocial factors that can

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be incorporated into culturally-sensitive behavioral interventions that are well received and easy to implement to improve Latina patients' treatment adherence.

(4) The Importance of the Research to Patients with Breast Cancer – Not adhering to medical treatment is very problematic among patients. In the case of cancer treatment, skipping or missing chemotherapy and radiotherapy can lead to poorer outcome and increased risk of recurrence or disease progression. [6,7] A third of breast cancer patients do not adhere properly to their treatment. [7] For Latina patients, initiation of treatment tends to be delayed longer, along with compromised adherence to follow-up treatment.[4] Lack of treatment adherence can lead to disparities in disease progression and breast cancer mortality for these women. While a variety of reasons for poor treatment adherence exist,[8] there is a lack of information about how behavioral factors and medical barriers influence Latinas' treatment adherence. If cultural, psychosocial, educational and language barriers to medical care play a role in treatment adherence, properly addressing these barriers through culturally-sensitive behavioral interventions could lead to improvement in Latinas' BC treatment adherence and completion. Although behavioral interventions are rarely implemented to improve adherence among breast cancer patients, [10] we have evidence that suggests that an intervention that decreases distress, improves symptom management and increases cancer-specific knowledge leads to increased treatment adherence and completion among Latinas and potentially increase survival outcomes.[11]

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PI Name: M. Tish Knobf, PhD

Institution: Yale University

Mechanism: Post-Baccalaureate Training in Disparities Research

Application Title: Interdisciplinary research training in breast cancer disparities

Abstract

The death rate for African American (AA) women with breast cancer is significantly higher than White women. This disparity can be explained by differences in tumor biology, economic and social factors related to access to care, lifestyle behaviors and increased risk of other illnesses such as hypertension, diabetes and heart disease. Over the past decade, AA with breast cancer have been identified with certain biologic tumor characteristics associated with a higher risk for recurrence and AA women of lower educational and economic levels often do not engage in routine mammography and may be diagnosed at later stages. Overweight, obesity and sedentary behaviors in AA women may further contribute to adverse outcomes for breast cancer and all cause mortality. Finally, there are few minority providers and researchers in breast cancer research. The purpose of this training program is to teach and mentor students recruited from medicine, nursing, and public health to participate in research that addresses breast cancer disparities. The training goals are to (1) increase trainee's knowledge about African American (AA) women diagnosed with breast cancer, (2) provide research knowledge and skills and (3) identify and conduct a research project related to breast cancer disparities with established breast cancer research mentors. The Yale Cancer Center is a designated National Cancer Institute Comprehensive Cancer Center and provides a rich and diverse clinical and research environment. This training program will use course content that currently exists in the master's and doctoral programs of the Yale Schools of Nursing (YSN), Medicine (YSM), and Epidemiology and Public Health (EPH). The Yale School of Nursing (YSN) has a master's oncology course, EPH has a Chronic Illness Epidemiologic focus and both YSN and EPH have doctoral programs with faculty who conduct breast cancer research. Course content will be tailored to meet the needs of the individual trainee (educational level [master's versus doctoral] previous experience and interest) and also will be enhanced by a mentored research experience specific to the trainee's unique goals. The specific aims of the program for the trainee are (1) to explore factors that are associated with clinical outcomes of AA women with breast cancer (2) to conduct appropriate analyses, and (3) to disseminate findings in the form of presentations and publications. The importance of the training program for AA women with breast cancer is to recruit minority students as clinical providers and researchers into breast cancer research. Two ongoing studies of Drs. Harris and Knobf will provide data for trainees to consider for their research project. The purpose of Dr. Harris' study is to analyze the molecular profiles of tumors in AA women with breast cancer who have received primary (mastectomy with or without reconstruction, or breast conservation surgery with radiation) and/or adjuvant chemotherapy. The hypothesis is that minority women have changes in some breast cancer genes that may result to poorer clinical outcomes, such as increased risk of recurrence and decreased survival. The specific aims are to (1) to explore factors associated with clinical outcomes of AA women with breast cancer through a retrospective medical chart review of 600 AA breast cancer patients and (2) to analyze tumor tissue in a sub-sample of 100 AA compared to 100 Caucasian women for gene expression and unique

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biomarkers. The importance of this research to patients with breast cancer is to further explain why there are different outcomes for AA. The medical record review will provide information to assess factors that contribute to outcomes for AA women and if there are differences between mastectomy and breast conserving treatments. If unique genes are identified, they offer a potential as a target for treatment aimed at decreasing disparities. Dr. Knobf has a 6 week psycho-educational intervention focused on physical activity and healthy eating in AA breast cancer survivors. This is a descriptive longitudinal program intervention. Using standardized instruments, demographic, breast cancer knowledge, symptoms, quality of life and healthy lifestyle behavior data are collected at baseline, end of program and 3 and 6 months after the program intervention. The hypothesis is that women who attend the program will adopt positive healthy lifestyle behaviors that will contribute to a better quality of life and contribute to reducing the woman's risks for breast cancer recurrence and also for risks of other health conditions prominent in AA women, such as diabetes and hypertension. The importance of this research for AA women with breast cancer is to increase awareness of the importance of healthy eating and physical activity on clinical outcomes and that there are activities that women can do to keep themselves healthy.

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PI Name: Christine Daley, PhD

Institution: University of Kansas Medical Center

Mechanism: Post-Baccalaureate Training in Disparities Research

Application Title: American Indian Health Research & Education Alliance Master in Public Health Program in American Indian breast cancer disparities

Abstract

American Indians die from breast cancer more often than any other racial/ethnic group; they have death rates twice that of white women. A major reason for this high death rate is that breast cancer is diagnosed more often at later stages than for other racial/ethnic groups. Almost one-half of all breast cancer cases diagnosed in American Indian women are diagnosed at late stages, with regional or distant spread of disease. One of the major reasons for late stage at diagnosis is low rates of mammography among American Indian women. Only 37% of eligible American Indian women report having a mammogram in the last year and rates of mammography are declining. Our own research in Kansas and Missouri shows us that American Indian women in our region have similar rates of mammography to other American Indian women around the country and would benefit from improved education and programs.

Even though American Indian women have very poor outcomes from breast cancer, little research has been done with them to improve early diagnosis and survival. In addition, few American Indians go into the health professions to treat breast cancer and few go into breast cancer research. We propose the development of a program within our Master's in Public Health (MPH) program focused on American Indian breast cancer disparities to begin to train American Indians in breast cancer research, particularly prevention and screening through mammography. Further, we will sponsor the first three students in the program, all of whom will be of American Indian descent and will be chosen based on their desire to conduct breast cancer research in their communities. The University of Kansas Medical Center has an MPH program where students can choose to focus on health disparities research. When a student focuses on health disparities, he or she can also choose to focus specifically on American Indians through our Program in American Indian Community Health, led by the Principal Investigator on this project, Dr. Christine Daley. Within this track, we plan to create a focus on breast cancer disparities through additional classes and practical experience working with Dr. Daley's research team. We anticipate many of our potential students will come from one of our partner institutions, Haskell Indian Nations University. Dr. Daley and Dr. Won Choi (co-Investigator on this project and Executive Director of the MPH Program) have worked with Haskell University over the past year and one-half to create a new major in community health. Drs. Daley and Choi are currently teaching in the major and can recruit students directly from their classes into our MPH program.

Dr. Daley has been working with American Indian communities since 1995 and has conducted several breast cancer studies during that time, all focused on prevention and mammography. Most recently, she completed a needs assessment in Kansas and Missouri to understand needs and barriers related to mammography for American Indians in the region, thanks to funding from Susan G. Komen for the Cure. During the time she

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conducted this research, she created a partnership with several American Indian Nations, community organizations, and colleges/universities, all focused on reducing health disparities among American Indians. This alliance has become known as the American Indian Health Research and Education Alliance (AIHREA) and has formed a strong research group that includes participation of community members during all parts of the research. Based on Dr. Daley's needs assessment, AIHREA and the Program in American Indian Community Health at the University of Kansas Medical Center are now developing and testing several different programs for the local community, including the following: (1) the development of a mobile mammography program for reservation communities; (2) the development of a transportation system for urban American Indian communities to get to mammography facilities; (3) a one-day traveling breast cancer education program with mammography; (4) culturally-tailored educational materials that also provide specific information about mammography in Kansas and Missouri; (5) a culturally-tailored program where American Indian elders act as "patient navigators" to guide women through mammography and follow-up for positive results, when necessary; and (6) a study of women's satisfaction with mammography to help determine why American Indian women are less likely to get a second mammogram after having had one. Students in our MPH in American Indian Breast Cancer Disparities will be able to choose one of these projects on which to work as a part of their degree.

The new AIHREA MPH in Breast Cancer Disparities will train new AI researchers in breast cancer prevention research and program development and testing. It will enable them to begin to conduct research that will directly benefit their communities. Our ongoing research, opportunities through our MPH program, and relationship with the new community health major at Haskell Indian Nations University present a unique and ideal location to train AI researchers in breast cancer disparities.

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PI Name: Karen Freund, MD

Institution: Boston University School of Medicine

Mechanism: Post-Baccalaureate Training in Disparities Research

Application Title: Boston University Women's Health Unit Training Program: patient navigation and clinical intervention research to eliminate breast cancer health disparities

Abstract

We know that women with breast cancer who are poor, who have no insurance or are underserved, or who are from racially and ethnic minority populations are more likely to die from their breast cancer, even if they receive mammography and other breast cancer screening. These disparities in survival are seen in all stages, and all types of breast cancer, suggesting this is not due to biological differences, but due to something about how we deliver health care.

There is recent great interest in patient navigation as a means of support in the care of patients with cancer, and for the patients undergoing diagnostic tests for a possible breast cancer. Our research group is interested in studying whether patient navigation, in providing additional support throughout the diagnosis and treatment process, can improve the outcomes of minority and low income women with breast cancer. We have designed and implemented a patient navigation program in 6 community health care centers in Boston Massachusetts. We have enrolled 3200 women into our study; the majority are low income, minority or immigrant women, with no health insurance or public health insurance. We have completed enrollment, and are now preparing to begin the analysis phase of our research.

We have also conducted over 16 observations of 6 patient navigators, at our sites and 8 other sites nationwide. These are the first structure observation studies to understand how navigators accomplish their goals for their patients.

Lastly, our research program has conducted our research study in the middle of a "natural experiment" of Massachusetts Health Insurance Reform. We collected baseline data on 1000 women with abnormal cancer screening tests in 2004 - 05 before insurance reform. Our study was conducted in 2007-9, the year after Health Insurance Reform was past in the legislature and enacted. Our population of patient in our community health centers experienced real changes in their care as a result of insurance reform - for example, over 20% of our patients were now insured under our public option, state subsidized insurance plan. We are one of the few research studies able to now examine the effect of health insurance reform, by specifically looking at its effect on women with breast cancer.

Our research, conducted in the Women's Health Interdisciplinary Research center at Boston University, has focused our research on health disparities in women. We have as a key goal of our research center training at all levels. We look forward to using the resources of this important study to train three baccalaureate graduates, and mentoring them in careers in health disparities addressing breast cancer.

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The research questions we will be answering are:

- 1) Does patient navigation reduce the time to finding out whether one has cancer after an abnormal mammogram or other screening study?
- 2) Does patient navigation reduce the time to completing cancer treatment for women who are diagnosed with breast cancer
- 3) Do women who receive patient navigation services report more satisfaction with their care? And less stress during the process?
- 4) What are the activities that navigators do to support their patients?
- 5) Who are the people that navigators work with in order to support their patients?
- 6) Can we learn best practices, those activities and resource people that make patient navigator most effective?
- 7) Did health insurance reform result in fewer women having gaps and changes in their insurance during breast cancer diagnosis or treatment?
- 8) Do women who have fewer insurance gaps receive better care?
- 9) Do women who have gaps in insurance benefit from the resources of a patient navigator?

Our training program will provide 3 individuals the opportunity to gain from coursework on public health and outcomes research, while receiving hands-on training as part of the analysis of the results of this critical study. All trainees will work closely with two mentors, Dr Karen Freund and one of the collaborators of the program. Each will be assigned to work with the research team on one or two of these main outcomes. They will participate in meeting with our community advisory board, and learn the principles of community based participatory research. They will do analyses under supervision. They will present their findings at national meetings.

All trainees will apply for a degree program at Boston University School of Public Health. They will complete the course work. Each will have a thesis requirement for their degree program. Each will work with the mentors in the program and formulate his or her own hypothesis, study design and analysis on some aspect of the study. They will complete their thesis, and submit this for presentation at national meetings and for publication as first author.

At the end of the training, we anticipate having trained 3 new health disparities investigators, who have developed skills and understanding to conduct research in health disparities, able to interact and design intervention with community participation, and with an understanding of the broader social determinants of health.

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PI Name: Roger Anderson, PhD

Institution: Pennsylvania State University College of Medicine

Mechanism: Post-Baccalaureate Training in Disparities Research

Application Title: Breast cancer disparities in rural Appalachia

Abstract

The objective of the proposed training program is to mentor and support student research aimed at describing, understanding, and ultimately reducing disparities in access to breast cancer screening and treatment and related health outcomes in rural Appalachia. To accomplish this objective, the program has four major training goals:

1) Students will gain an in-depth understanding of the epidemiology of breast cancer in the U.S. overall and patterns of disparities in breast cancer screening, treatment, and outcomes in rural Appalachia, and be able to identify potential mechanisms underlying these disparities. Attainment of this goal will be demonstrated through satisfactory performance in a new course focusing specifically on research related to breast cancer disparities and a second course that examines what is currently known about socioeconomic and race/ethnic disparities in health and health care.

2) Students will develop skills in critical thinking and research methods applicable to research in breast cancer disparities. Attainment of this goal will be demonstrated through satisfactory performance in the courses described above as well as other coursework that provides a comprehensive overview of relevant social scientific research and statistical methods.

3) Students will complete a community-based internship with the Appalachian Community Cancer Network (ACCN) focused on disparities in breast cancer.

4) Students will develop a final research paper containing original empirical findings focused on breast cancer disparities in Appalachia and specify implications of the findings for disparity reduction. Attainment of this goal will be demonstrated through production of a final research paper and dissemination of the research findings through presentations at scientific meetings and submission of papers for publication in scholarly journals.

Breast cancer is of particular research interest in Appalachia because it is the most prevalent cancer among women, has a good prognosis when treated with guideline recommended care, and yet shows disparities in outcomes compared with other regions of the country. Women in the Appalachian region as a whole have relatively high risks of mortality from breast cancer. For example, central Appalachia once ranked 42nd highest in breast cancer mortality rates in the U.S., and currently it ranks 2nd. Reasons for the persistence of elevated breast cancer mortality risk in Appalachia despite medical and scientific advances are likely to include a range of factors, but it is hypothesized that barriers to optimal screening and management posed by lack of regular access to primary care and to comprehensive oncology services will be of paramount importance.

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Students in the proposed training program will design and carry out research studies that examine key factors that are likely to contribute to persistent disparities in cancer stage, treatment patterns and outcomes in Appalachia. Factors that will be examined include social and economic conditions of families and communities, the supply of health care providers and facilities available, the distance that women must travel to reach treatment centers, the level of community awareness about the importance of cancer screening, and the degree to which communities invest resources in promoting the prevention of breast cancer. An undersupply of effective community services to facilitate health promotion, primary care coordination, and comprehensive cancer services in the region may contribute to the total excess burden of cancer in Appalachia across the spectrum – from cancer screening through survivorship. The research that will be conducted by students in the training program in collaboration with their faculty mentors will produce important new information that can be used to improve strategies for breast cancer prevention, control, and treatment.