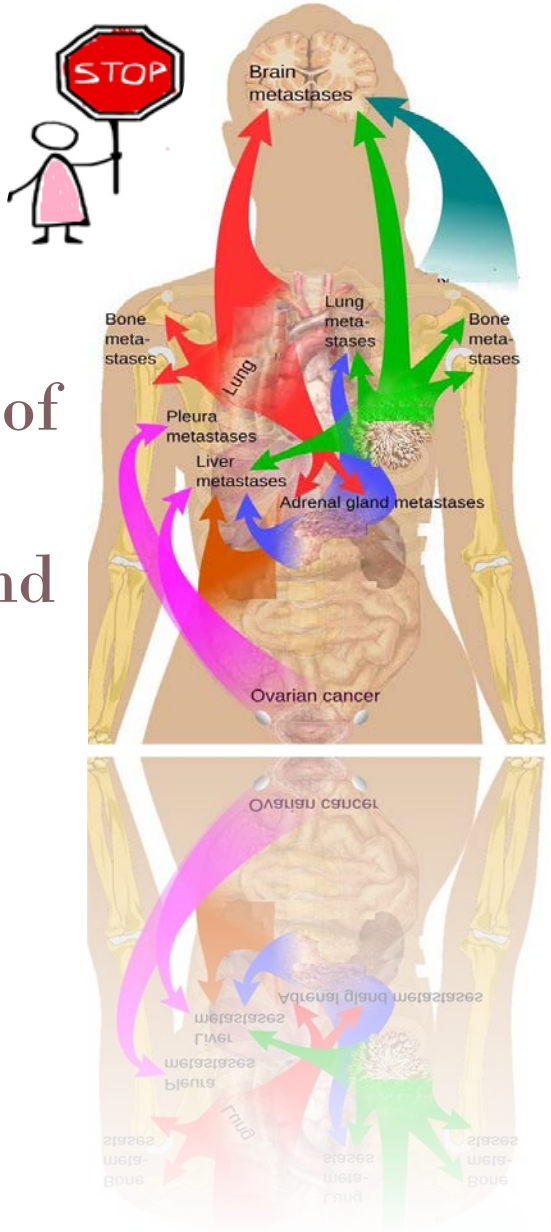
The Fourth Annual SBCC Breast Cancer Research Retreat



Honoring the contributions of The Carol M. Baldwin Breast Cancer Research Fund

October 6, 2017 | Hilton Garden Inn

Stony Brook Cancer Center

Breast Cancer Research Retreat
October 6, 2017
Hilton Garden Inn, Stony Brook NY

12:00 – 1:00pm **Lunch and short talks from the 2017 Carol M. Baldwin Award Recipients**
Michael Frohman, PhD – Presentation by Eric Roth, BS
Kenneth Shroyer, MD – Presentation by Stanislaus Wong, PhD
Chung Huang, PhD – Presentation by Jie Ding, MS
Natalia Marchenko, PhD
Hyungjin Kim, PhD

1:00 – 1:50pm **Keynote Lecture "*ECM and Cellular Heterogeneity in Breast Cancer*"**
Peter Kabos, MD, Associated Professor & Deputy Director
Breast Cancer Research Program, University of Colorado Cancer Center

1:50 – 2:00pm **Coffee Break**

2:00 – 3:00pm **Session I Presentations from Past Baldwin Awardees**

Single Cell RNA-Seq Analysis of Breast Cancer Progression
Ana Paula Delgado graduate student Dr. Scott Power's Laboratory

p63 promotes pregnancy-associated HER2-positive breast cancer in vivo, via maintaining parity-identified mammary epithelial cells (PIMECs)
Evguenia (Jenya) Alexandrova, PhD

RNF8 is a new drug target for basal-like breast cancer
Lori Chan, PhD

High resolution in vivo imaging of breast cancer metastasis
Benjamin Martin, Ph.D.

3:00 – 3:20pm **Coffee Break and Group Photo**

3:30 – 4:30pm **Session II Clinical Research Opportunities in Breast Cancer**

Contrast Enhanced & Wide-angle Digital Breast Tomosynthesis
Paul Fisher, MD

The Microenvironment in Breast Cancer Prevention
Alison Stopeck, MD

Like Oil and Water: Nanoemulsions, Polyunsaturated Fatty Acids, Next-Generation Taxoids and Breast Cancer
Jules Cohen, MD

Optimizing Cardiac Care for Oncology Patients: A model of a multi-disciplinary approach to cardio-oncology
Lea Baer, MD

4:30 – 5:30pm **Poster session with light refreshments**

Stony Brook Cancer Center

Greetings from the Director

Once again, I take this opportunity to welcome you to now our fourth Breast Cancer research retreat for the Stony Brook Cancer Center.

This year we are recognizing the impact of The Carol M. Baldwin Breast Cancer Research Funds on our breast cancer research efforts. Since 2008, this fund has provided support for 36 new breast cancer research initiatives including 5 newly awarded research efforts that you will learn about over the noon hour. Today, you will gain an overview of the scope and significance of the breast cancer research effort at the Stony Brook Cancer Center. My hope is that the retreat inspires collaborations and interactions within our expanding breast cancer research scientific community.

In addition to highlighting the impact of the Carol M. Baldwin Breast Cancer Research Funds, Session 2 of the day will focus on our clinical breast cancer research. This session is designed to highlight important patient-centered, clinical research at our center in early detection, prevention, treatment and survivorship. I am excited by this session as it illustrates our expanding breadth of effort in breast cancer clinical research. I am looking forward to a robust discussion on collaborative opportunities and furtherance of this effort at our center.

Looking at the presentations and numbers of registrants for the retreat and poster session, I am proud to see the impacts of our investments and successful expansion of the number of researchers working in the breast cancer domain. However, as shown on the cover photograph of this booklet, there remain significant challenges to our patients across their cancer care continuum with cancer metastasis remaining a threat to our patients. Today, I hope you will be energized in your efforts to tackle these challenges as the Cancer Center continues to expand through the support of the Carol M Baldwin Breast Cancer Research Fund and many other local and national foundations.

With warm regards,

Yusuf Hannun, MD

Program Committee

Patricia Thompson, PhD
Department of Pathology

Jules Cohen, MD
Department of Medicine
Division of Hematology/Oncology

Alison Stopeck, MD
Department of Medicine
Division of Hematology/Oncology

Lea Baer, MD
Department of Medicine
Division of Hematology/Oncology

Acknowledgments

Thanks to all speakers and faculty for their enthusiastic participation.

Special thanks to Ms. Lauren Cutaia and Ms. Megan Fitzgerald for their time and efforts in organizing this event.

2017 Carol M Baldwin
Breast Cancer Research Fund

Awardee Presentations

Metastatic and Metabolic Roles of Phospholipase D in Breast Cancer

Presented By: Eric Roth, MD, on behalf of Michael Frohman, MD

Breast cancer is the most common female malignancy worldwide, affecting approximately 1 in 8 women. The formation of chemo-resistant metastasis, which is almost inevitably lethal, makes breast cancer the second-leading cause of cancer-related deaths in the United States. For the past twenty years, the two classical isoforms of phospholipase D (PLD) have been studied and reported to regulate multiple oncogenic traits and pathways through generation of phosphatidic acid (PA). Recent studies have linked PLD to regulation of metabolic pathways including autophagy, lipid droplet formation, and release of fatty acids for oxidation. Although PLD1 and PLD2 both produce PA, differences in their subcellular localization results in each having isoform-specific regulatory roles. However, some of these pathways elicit compensatory mechanisms when only one isotype is inhibited or ablated. My research aims to determine the effects of PLD1/2 double knockout in breast cancer metastasis and metabolism.

Notes

Multifunctional Nanomaterials for Breast Cancer Theranostics

Presented By: Stanislaus Wong, MD, on behalf of Kenneth Shroyer, MD
Collaborative project by Kenneth Shroyer, MD, Stanislaus Wong, PhD and Stella Tsirka, PhD

Nanomaterials have emerged as powerful tools to enhance detection capabilities, but can also be engineered to cross biological barriers, thereby enabling earlier diagnosis and more effective treatment. It was recently reported that core-shell nanomaterials carrying a chemotherapeutic and a photosensitizer were able to target and ablate cells in a model of colorectal cancer and to activate the immune response in the environment of the tumor. Here we will synthesize a novel family of metal-oxide-based core-shell nanomaterials. The nanoparticles' chemical composition renders them biocompatible and with hyperthermic potential, which can be used as a therapeutic modality against the tumor cells. We will use a model of breast cancer induced by the inoculation of EO771 cells into a mammary gland of immunocompetent mice. The nanoparticles will be conjugated with Rhodamine B (RhB). We have previously reported that the nanoparticles can be taken up by innate immune cells, such as microglia, the innate immune cells of the CNS, or macrophages, which have been shown to migrate to and associate with the tumors. After sessions of hyperthermia, we will assess at various time points toxicity and tumor size, as well as the immune environment around the tumor. We anticipate microglia/macrophages will effectively 'carry' the nanoparticles to the tumor where the hyperthermia will occur. If successful, the nanoparticles could be used not only as compounds that can be used to treat breast cancer but also as non-invasive diagnostics, given the paramagnetic (MRI-appropriate) properties of the nanoparticles.

Notes

Early treatment response detection using accurate and reproducible breast density measure derived from MRI

Presented By: Jie Ding, MS, on behalf of Chuan Huang, MD

Synopsis: Increased breast density (BD) is a significant independent risk factor for breast cancer. BD has emerged as a potential modifiable risk factor with change in BD increasingly incorporated as an intermediate surrogate endpoint to evaluate efficacy of drugs in breast cancer treatment and prevention. Based on fat-water decomposition MRI technique, we propose an optimized automated, highly reproducible breast density measurement, which is free of ionizing radiation and directly comparable to mammographic density. This method enables the possibility of early detection of small breast density changes in clinical practice. The immediate goal is to apply our new MRI derived BD as a biomarker in research studies aimed at assessing the action of candidate prevention compounds on BD at an earlier time point than what is currently achievable using conventional mammography.

Notes

Translational significance of mutant p53 in Her2-positive breast cancer

Presented By: Natalia Marchenko, PhD Assistant Professor of Pathology

Ample clinical data support the notion that mutations in the p53 gene with consequent stabilization of mutant p53 (mutp53) proteins are potent driving events in Her2 (ErbB2) breast cancer. Yet, no systematic studies have been done in clinically relevant *in vivo* models to assess translational significance of mutp53 in Her2 breast cancer therapy. While a number of clinical studies report chemoresistance of mutp53 mammary tumors, alternative treatment protocols have not been developed in a clinic specifically for mutp53 harboring patients.

Our previous *in vitro* and *in vivo* studies established novel oncogenic function of mutp53 in Her2 breast cancer. We found that mutp53 via mutp53-HSF1/Hsp90-ErbB2 feed-forward loop promotes Hsp90-mediated stabilization of ErbB2 and amplifies ErbB2 signaling. Targeted interception of this feed-forward loop by the ErbB2 inhibitor lapatinib destabilizes mutp53. Here we seek to investigate the translational aspect of these findings.

Based on our preliminary studies, we hypothesize that mutp53 predicts higher sensitivity to Her2-targeted therapies in mutp53;ErbB2 cancers via two complementary mechanisms- amplification of ErbB2 signaling and destabilization of mutp53. This proposal seeks to investigate whether the mutational status of p53 has a translational value in predicting therapeutic response to ErbB2 targeted therapies. We will assess the impact of mutational status of p53 on the efficacy of ErbB2-targeted therapies *in vitro* (Aim 1a), *in vivo* in genetically engineered mouse model for mutp53;ErbB2 cancer (Aim 1b) and human retrospective clinical study correlating mutational status of p53 with clinical responses to Her2-targeted therapies (Aim 2).

Overall, this project will immediately yield important information to improve diagnostic and treatment options for Her2 breast cancer patients with regard to mutational status of p53.

Notes

Mechanisms of DNA replication fork protection in breast cancer

Presented by: Hyungjin Kim, PhD, Department of Pharmacological Sciences

Hereditary mutations in BRCA1 and BRCA2 (BRCA1/2) genes disrupt DNA double-strand break repair and predispose carriers to breast cancer. BRCA1/2-deficient breast cancer cells also exhibit increased replication stress, which has been exploited for the development of Poly (ADP-ribose) polymerase (PARP) inhibitor (PARPi) to specifically kill cancer cells with BRCA1/2 mutations. However, its efficacy is limited by several resistance mechanisms including induced replication fork stabilization. Thus, understanding the mechanisms by which BRCA1/2-deficient tumors mitigate the elevated replication stress for their survival and how their strategies are exploited for drug resistance are essential for improving therapeutic outcomes. In this application, we will identify mechanisms by which the replication stress response is regulated in breast cancer. Specifically, we will test the hypothesis that SDE2, a genome surveillance factor that we previously identified at replication forks, exerts a fork protection mechanism to counteract the replication stress of BRCA1/2-deficient cancer cells and modulates the chemotherapeutic response to PARPi. Together, this study is expected to provide new insights into the BRCA1/2-dependent fork protection mechanism and help design predictive biomarkers and therapeutic targets for the treatment of breast cancer.

Notes

Keynote

Stroma, ECM and Cellular Heterogeneity in Breast Cancer

Peter Kabos, MD
Associated Professor & Deputy Director, Breast Cancer Research Program,
University of Colorado Cancer Center

The most common subtype of breast cancer is estrogen receptor (ER) positive. Targeting ER is an effective therapy, but development of anti-endocrine resistance remains a major cause of treatment failure. Attempts to uncover and therapeutically target mechanisms of anti-endocrine resistance have focused primarily on tumor intrinsic traits, including tumor initiating cells. Our laboratory is focused on model development and study of the role of tumor microenvironment and host specific factors influencing treatment resistance and disease progression. We have recently identified two subtypes of cancer-associated fibroblasts (CAFs), based on their CD146 expression. We have shown that CAF subtypes differentially contribute to tumoral ER expression and tamoxifen sensitivity. The $CD146^{neg}$ CAFs enforce ER independent growth, mediate tamoxifen resistance and drive disease progression. Our novel tools and models will allow us to study the tumor cells and their microenvironment in appropriate context with the goal of developing novel treatment strategies and improving patient outcomes.

Notes

Session 1

2017 Carol M Baldwin
Breast Cancer Research Fund

Awardee Research Updates

Single Cell RNA-Seq Analysis of Breast Cancer Progression

Authors: Ana Paula Delgado, PhD, Alice Nemajerova, PhD, Ute Moll, MD, Mikala Egeblad, PhD, Jinyu Li, PhD & Scott Powers, PhD

Presented By: Ana Delgado, PhD - Graduate student, Dr. Scott Powers Lab

We have used single cell RNA-seq analysis to examine over 20,000 cells from different stages of disease progression using the MMTV-PyMT model for luminal B breast cancer. In addition to changes in mammary epithelial cells, we observed pronounced accumulation of several non-tumor cell types during progression, including T-lymphocytes, macrophages, endothelial cells, and cancer-associated fibroblasts. Cluster analysis suggests that cancer-associated fibroblasts evolve into three distinct subtypes with different functions: extracellular matrix production, immunomodulatory, and contractility. Further application of this technology should yield additional insights and lead to development of novel therapeutic strategies.

Notes

p63 promotes pregnancy-associated HER2-positive breast cancer *in vivo*, via maintaining parity-identified mammary epithelial cells (PIMECs)

Authors: Evguenia Alexandrova, PhD, Alisha Yallowitz, PhD, Flaminia Talos, MD, Sulan Xu, Research Support Specialist, Natalia Marchenko, PhD, Ute Moll, MD

Presented By: Evguenia Alexandrova, PhD Department of Pathology, Stony Brook University

Emerging clinical data suggest that although pregnancy reduces the *overall* life-long risk of breast cancer, it seems to specifically promote HER2-positive breast cancer, which is one of the deadliest subtypes and is characterized by *HER2* (human EGF receptor 2) gene amplification (aka ErbB2/Neu in mice). Consistently, pregnancy accelerates the onset of ErbB2/Neu-driven breast cancer in mouse models, which is associated with the post-partum accumulation of PIMECs (parity-identified mammary epithelial cells), a known tumor-initiating cell of ErbB2/Neu breast cancer in mice. Using the MMTV-ErbB2 *in vivo* genetic mouse model of HER2-positive breast cancer, we found that the post-partum PIMEC accumulation and HER2-positive breast cancer both require the epithelial master regulator p63, a homologue of the p53 tumor suppressor. Thus, in p63 heterozygous females (p63+/-; p63-/- animals die at birth), the post-partum PIMEC content is reduced by 30% ($p=0.01$), breast cancer onset is delayed by >3 weeks ($p=0.01$) and the overall survival is extended by >6 weeks ($p<0.001$), compared to p63+/+ littermates. This is not observed in virgin animals. Mechanistically, p63 maintains post-lactation PIMEC content by suppressing the pro-apoptotic IL6/pStat3 signaling and promoting the pro-survival Neuregulin/pStat5 signaling. Whether a similar p63/PIMEC-dependent mechanism of pregnancy-associated HER2-positive breast cancer takes place in patients remains to be identified.

Notes

RNF8 is a new drug target for basal-like breast cancer

Authors: Hong-Jen Lee, Diane Ruan and Lori Chan, PhD

Presented By: Lori Chan, PhD

Basal-like breast cancer (BLBC) is a heterogeneous subtype that harbored enriched cancer stem cell (CSC) populations in tumors. Conventional chemotherapy is used as a systematic treatment for BLBC at the time, but it spares the CSC population, which is known to contribute to cancer recurrence after the initial treatment. Therefore, identification of the core molecular pathway that control CSC activity and expansion is important for the development of effective cancer therapeutics for BLBC. Ubiquitination (Ub) is a versatile regulatory signal. In contrast to the canonical K48-ubiquitination (Ub) pathways that have been known to lead to protein degradation, K63-Ub is a newly recognized regulatory post-translational modification important for activating protein function and its mediated signal pathways. Our previous work has established that K63-Ub activates Twist. Twist is a transcription factor not only generates CSCs from differentiated cancer cells through EMT. Through systematic screen, we identified RNF8 as a novel Twist activator by promoting its K63-Ub. By suing a series of complementary approaches including biochemical studies, preclinical animal models and clinical specimens, we will define RNF8's regulation in CSC regulation and CSC-mediated drug resistance in tumors. This work provides new strategy for the development of CSC-targeted therapy. This work is supported by National Institute of Health (5 K22 CA181412) and TRO Carol M. Baldwin Award.

Publications/Awards resulting from this work · Lee HJ, Li CF, Ruan D, Powers S, Thompson PA, Frohman MA, Chan CH*. The DNA damage transducer RNF8 facilitates cancer chemoresistance and progression through Twist activation (2016) Molecular Cell (Featured Article) 63: 1021–1033 Research highlight by Krista L. Bledsoe (2016) Cancer Discovery

Ruan D, He J, Li CF, Lee HJ, Liu J, Lin HK and Chan CH* (2017) Skp2 deficiency restricts the progression and stem cell features of castration-resistant prostate cancer by destabilizing Twist. Oncogene 6:4299-4310

Research highlights by Annette Fenner (2017) Nature Reviews Urology

Grant funding: NCI R01 and Komen CCR grants

Notes

High resolution in vivo imaging of breast cancer metastasis

Authors: David Matus, PhD and Benjamin Martin, PhD Collaboration

Presented By: Benjamin Martin, PhD

We are using a zebrafish xenograft system to analyze human breast cancer metastasis in vivo and at high resolution. The transparent zebrafish embryo, along with new adaptive optics light sheet microscopy technology, provides a platform for visualizing the cellular and subcellular dynamics of breast cancer extravasation. Here we present data that supports a model in which breast cancer cells coopt cellular behaviors utilized by leukocytes during extravasation.

Notes

Session 2

Breast Cancer Clinical Research Opportunities

Cancer Imaging

Contrast Enhanced and Wide-angle Digital Breast Tomosynthesis

Presented By: Paul Fisher, MD

We are conducting breast imaging studies on iodinated contrast enhanced mammography and wide-angle digital breast tomosynthesis. The goal is to improve cancer detection in women with dense breast, and the identification of malignant breast lesions prior to biopsy. We will present the motivations for these clinical studies, and examples of clinical results.

Notes

Prevention

The Microenvironment in Breast Cancer Prevention

Presented By: Alison Stopeck, MD

Anti-angiogenics, immunotherapy, and bone-modifying agents are examples of current therapies targeting the microenvironment that have become successful for the treatment of metastatic cancer. The microenvironment is not just permissive, but can also foster cancer progression and development. In my talk, I will discuss the role of the NSAID, sulindac, in primary breast cancer development via its effects on the stroma and density of breast tissue.

Notes

Treatment

Like Oil and Water: Nanoemulsions, Polyunsaturated Fatty Acids, Next-Generation Taxoids and Breast Cancer

Presented By: Jules Cohen, MD

Metastatic breast cancer is incurable with only 20% of patients surviving past 5 years. Cancer cells develop taxoid resistance through multidrug-resistance (MDR) efflux pumps that prevent intracellular accumulation of cytotoxic levels of drug. Next-generation taxoids have been designed to avoid resistance but suffer from low solubility and high systemic toxicity. Conjugation of taxoid to polyunsaturated fatty acids such as docosahexaenoic acid (DHA) facilitates selective delivery to tumor cells and reduces systemic doses necessary for tumor response. Nanoemulsions (NE) of these drug-small molecule conjugates increase their solubility and demonstrate better delivery to the cancer cell as well as enhanced permeability and retention. The Ojima lab, in collaboration with the biotech company Targagenix, has developed a candidate molecule currently named NE-DHA-SBT-1214. Unconjugated next-generation taxoid SBT-1214 is more effective than paclitaxel against MDR breast cancer cell lines in animal models. SBT-1214 shows particular efficacy against cancer stem cells as well. DHA-conjugated SBT-1214 kills breast cancer cells at lower concentrations than unconjugated SBT-1214. Nanoemulsion of DHA-SBT-1214 improves its manufacturing yield, solubility and delivery to the cancer cell. Animal toxicity studies are currently underway in support of application to the FDA for Investigational New Drug (IND) status. We are currently planning a first-in-human phase I trial of this exciting new drug with Stony Brook as the lead clinical site.

Notes

Survivorship

Optimizing Cardiac Care for Oncology Patients: A Model of a Multidisciplinary Approach to Oncology

Presented By: Lea Baer, MD

Cardiotoxicity of highly effective chemotherapeutic regimens pose a significant challenge to cancer patients and to their quality of life. This is particularly true for breast cancer patients who are achieving cures and long durable relapse free survival times from the use of these agents. Current understanding of the importance of cardiac care in the oncology patient, the important role for a multidisciplinary approach to cardiac management in cancer survivors and areas of research investigation will be presented and discussed.

Notes

Submitted Poster Abstracts

Early treatment response detection using accurate and reproducible breast density measure derived from MRI.

Authors: Jie Ding, Alison Stopeck, Yi Gao, Patricia A Thompson, Chuan Huang

Increased breast density (BD) is a significant independent risk factor for breast cancer. BD has emerged as a potential modifiable risk factor with change in BD increasingly incorporated as an intermediate surrogate endpoint to evaluate efficacy of drugs in breast cancer treatment and prevention. Based on fat-water decomposition MRI technique, we propose an optimized automated, highly reproducible breast density measurement, which is free of ionizing radiation and directly comparable to mammographic density. This method enables the possibility of early detection of small breast density changes in clinical practice. The immediate goal is to apply our new MRI derived BD as a biomarker in research studies aimed at assessing the action of candidate prevention compounds on BD at an earlier time point than what is currently achievable using conventional mammography.

Xq21.33 virus in breast cancer patients and cell lines

Authors: *1.*Michael H Dosik, MD, drdosik@gmail.com[1.], *2.*Rafael Contreras-Galindo, MD, rafaelc@med.umich.edu[2.], *3.*David M Markovits, MD, dmarkov@umich.edu[2.] and *4.*Mark H Kaplan, MD, mhkaplan@med.umich.edu[2.].

Institutions: [1]Private Practice/North Shore Hematology/Oncology Associates, Setauket, NY, United States, 11733 and [2]University of Michigan, Ann Arbor, MI, United States, 48109.

Background: Since the discovery of the Mouse Mammary Tumor Virus (MMTV) by Bittner in 1936, efforts have been made to find an analogous virus in humans. Human Endogenous Retrovirus-K (HERV-K) viruses are betaretrovirus-like and closely related to MMTV. The HERV-K (HML2) subgroup are the most recent (100,000-200-000 years ago) entries into the human genome and have less DNA inactivating damage than other HERVs, but nonetheless appeared to be defective. Both serologic and molecular biologic studies of HERV-Ks have yielded contradictory data in breast cancer without a major breakthrough regarding the etiology or management of human breast cancer. Recently, an intact unfixed HERV-K (HML2) provirus (Xq21.33) has been found with full open reading frames (ORFs) for all relevant viral genes, suggesting possible infectivity. This provirus is present in only a small fraction of the human population (<2.5%). We searched for Xq21.33 sequences in breast cancer cell lines and breast cancer patients, and other conditions.

Methods: We performed PCR testing of DNA from >30 breast cancers as well as other diseases and 12 breast cancer cell lines using primers for the gag and env genes of Xq21.33 and for the 5' and 3' side of Xq21.33 where the virus is integrated. This results in 1100 bp and 4300 bp products indicative of the presence of this virus.

Results: Of the >30 American breast cancer patients tested, no women with breast cancer were found to have this provirus. One African American HIV positive man with diffuse large B cell lymphoma had this provirus present. Of 12 breast cancer cell lines tested, only DT22 was positive for the Xq21.33 virus. **D**issociated **T**umor 22 is a basal/claudin-low, triple negative cell line, and is tumorigenic in the NOD/SCID mouse model. Several other cell lines including prostate cancer, melanoma and T-cell lymphoma derived lines were similarly tested and negative. Further studies are being carried out to see if viral particles are present from the cell line and if reverse transcriptase activity is expressed.

Conclusions: This preliminary finding leads to speculation about a possible pathogenetic role for Xq21.33 in this breast cancer cell line and more broadly, in breast cancer and other diseases. Studies are in progress to further elucidate this.

Inhibiting CDK4/6 rescues C. elegans anchor cell invasion devoid of nhr-67/tlx

Authors: Michael A. Martinez, Abraham Q. Kohrman, and David Q. Matus

Invasion and metastasis, both hallmarks of cancer, are traditionally viewed as pathologic processes in which uncontrolled cell proliferation and invasion occur simultaneously. Cell invasion, which is an active, dynamic process, not only occurs during cancer progression, but it also occurs during normal developmental events such as gastrulation, neural crest migration, and mammalian embryo implantation, as well as 'housekeeping' events such as immune surveillance and defense. Interestingly, due to the difficulty in examining this active, dynamic process *in vivo*, it is the least understood aspect of metastatic cancer. Preliminary studies in our laboratory show a functional link between G1 phase cell-cycle arrest and invasive behavior, clearly demonstrating that cell proliferation and invasion are mutually exclusive behaviors. We propose using *Caenorhabditis elegans*, a transparent nematode, as an *in vivo* experimental system to dissect cell invasive behaviors associated with metastatic cancers. *C. elegans* anchor cell (AC) invasion is a well-established *in vivo* model of cell invasive behavior. During normal larval development, the AC, originating in the uterus, invades the underlying vulval epithelium by traversing the basement membrane (BM). For the AC to invade the BM, it must exit the cell cycle in the G1 phase. The conserved nuclear hormone receptor, *nhr-67/tlx*, is a transcription factor required to maintain the AC in a G1 cell-cycle arrested state. Loss of this transcription factor results in mitotic ACs that fail to invade, forming a small non-invasive uterine tumor. Delivery of Palbociclib, a clinically used, small-molecule inhibitor of CDK4/6, results in cell-cycle arrest and BM invasion. Our experimental system, along with our studies in mammalian cells, will allow for unprecedented single-cell and subcellular resolution to study cell behaviors utilized during development that are hijacked by metastatic cancer cells.

Cell Cycle Regulation of the Invasions vs. Proliferation Decision

Authors: Robert Morabito, Abraham Q. Kohrman, Benjamin L. Martin and David Q. Matus
Stony Brook University Department of Biochemistry and Cell Biology

Uncontrolled cell proliferation and invasion through basement membranes are required for tumor formation, and metastasis respectively. In order for cancer cells to metastasize they must first be able to breakdown and invade basement membranes, a phenomenon traditionally thought to occur simultaneously with cell proliferation. Data from our lab, supported by recent breast cancer literature, shows a functional requirement for G1 phase cell-cycle arrest in basement membrane invasion, indicating basement membrane invasion and proliferation are mutually exclusive events. While the mechanisms of the cell cycle are well studied, how the control of cell cycle is linked to cell invasive behavior remains poorly understood, due to our inability to directly visualize precise cell cycle state in vivo during invasion. In order to visualize cell cycle state live, we have developed a CDK2 biosensor for use in multiple model systems, including but not limited to, zebrafish and mammalian cell culture. Our biosensor uses the dynamic cytoplasmic/nuclear localization of a portion of Human DNA Helicase B (DHB) linked to mNeonGreen to assess cell cycle state. The dynamic localization is the result of phosphorylation of the biosensor by CDK2. We have modified this sensor to allow for algorithmic assessment of cell cycle state. We are using this biosensor to quantify lineage specific differences between cycling cells, and to examine the dichotomy between proliferation and invasion.

Damon Runyon Cancer Research Foundation: DRR4714
Carol M. Baldwin Breast Cancer Research Fund: 1134629-1-72313

Identification of USP2 as a new therapeutic target in TNBC

Authors: Jiabei He[1], Diane Ruan[1], Hong-Jen Lee[1], Chia-Hsin (Lori) Chan[1]
[1]Department of Pharmacological Sciences, Stony Brook University, Stony Brook, NY 11790 USA

About 1 in 8 U.S. women will develop invasive breast cancer in their lifetime. Breast cancer is the second leading cause of cancer mortality for women. Hormonal and targeted therapeutics are useful in combating the growth of breast tumors with overexpressed estrogen, progesterone and/or Erb-B2 receptors. In contrast, around 15%-20% tumors that are lacking expression of these receptors, namely triple-negative breast cancer (TNBC), don't respond well to these existing therapeutics and consequently have poor therapeutic outcomes. It's therefore essential to identify new molecular targets and therapeutic approaches to tackle TNBC. Ubiquitin-specific protease 2 (USP2) is a deubiquitinating enzyme known to stabilizes protein substrates by removing their polyubiquitin chains. USP2 substrates include fatty-acid synthase (FAS), mouse double minute 2 homolog (MDM2), MDMX and cyclin D1, proteins that are associated with cancer progression. **Hypothesis**: Our bioinformatics analysis showed that USP2 gene expression is upregulated in around 20% of TNBC, leading us to study whether USP2 is a novel regulator of TNBC progression. **Results:** We used genetic approaches to silence USP2 expression in multiple TNBC cell lines. We found that USP2 knockdown inhibits breast cancer cells' abilities to migrate and invade. Moreover, tumor sphere formation assay demonstrated that USP2 is required for self-renewal of cancer stem cells. In addition, we discovered that USP2 affects the expression of epithelial–mesenchymal transition (EMT) markers in TNBC cells. Our future plan is to uncover the mechanism underlying how USP2 regulates EMT and TNBC. Together, our study suggests that USP2 is a promising therapeutic target in TNBC, and advances the knowledge how EMT is regulated. Source of Support: New York State Department of Health (DOH01-C31845GG)

High-throughput Genetic Interaction Screens in Breast Cancer Progression

Authors: Xiaoyu Zhao [1, 2], Scott Powers [1, 2]
Molecular and Cellular Biology program [1], Department of Pathology [2]
Stony Brook University, Stony Brook, NY 11794, USA

Cancer has long been known to be a multigenic disease, but many of the genetic interactions (GIs) underlying cancer progression are poorly studied. Combinatorial CRISPR screening makes it feasible to discover the GIs in a high-throughput mode. To unravel the multitude of GIs that lead to cancer, dual single-guide RNAs (sgRNA) libraries targeting 52 well-validated tumor suppressor genes (TSGs) were constructed and orthotopic injection of the cells expressing combinatorial CRISPR elements into nude mice will be applied for in-vivo GIs screens. The relative fitness of each sgRNA combination will then be estimated to define the GIs between two TSGs. The systematic discovery of genetic interactions in cancer progression is of great importance for understanding the mechanism of tumorigenesis and developing therapeutic strategies. The work is supported by National Cancer Institute and National Human Genome Research Institute.

Design of Inhibitors that Target Active ErbB2

Authors: Stephen Collins[1], Jiaye Guo[2], Robert Rizzo[2], W. Todd Miller[1]
Affiliations: 1. Physiology and Biophysics, Stony Brook University 2. Applied Math and Statistics, Stony Brook University.

The American Cancer Society estimates that about 246,660 new cases of invasive breast cancer will be diagnosed in women in the US this year. For 1 in 8 of these women, overexpression of the receptor tyrosine kinase ErbB2 is the driving force of the disease. In addition to ErbB2 overexpression, we have recently shown that several cancer-associated mutant forms of ErbB2 display increased kinase activity. There are currently no three-dimensional structure of the active form of ErbB2. Using computational methods, we predicted the structure of the active form of ErbB2. We used this model for large-scale docking (virtual screening) of a library containing ≈ 2 million compounds to identify potential inhibitors. Several of these predicted inhibitors showed activity against ErbB2 *in vitro*, suggesting that inhibitors can target the active form of the kinase. Source and Support: USAMRMC Breast Cancer Research Program grant # BC132617 Chemical Biology Training Program

Genome Engineering of Models to Study Copy Number Alterations

Authors: Manisha Rao, R. Scott Powers, PhD
Department of Pathology, Stony Brook Medicine

Copy number alterations (CNA) are multigenic in nature. Breast cancer has more oncogenic drivers altered by CNAs than single point mutations. We aim to develop cell models that mimic the structure of human breast tumor copy number alterations, using genome engineering tools. Crispr/Cas9 coupled with targeted integration of amplifiable minigenes will be used to generate cell models containing focal amplicons. These models would be able to target CNAs at varied genomic locus of interest and enable a better understanding of any induced selective dependencies. The multigenic CNA driven cell models will help lay the groundwork for identifying new therapeutic strategies. Funding sources: NCI, NIH, Carol M. Baldwin grant

CEACAM5 and SCUBE3: drivers that show dependencies in a subtype of breast cancer

Authors: Ada Mofunanya[1], Frank Celeste[1, 2], Jinyu Li[1], R. Scott Powers[1].
[1]Department of Pathology, [2]Graduate Program in Genetics Stony Brook University Stony Brook, NY 11794

We used cancer genome data from The Cancer Genome Atlas for the discovery and development of targets for human cancer therapeutics. Many of the best targets for cancer treatment are encoded by the genes that when altered drive cancer progression, and that when inhibited selectively block the survival of cancer cells with that particular alteration (linked driver-dependency, e.g. amplification of HER2 drives breast cancer development; amplified HER2 tumors are selectively dependent upon HER2 function). Following this paradigm, we performed functional genomic screening of aberrantly expressed candidate breast cancer genes along with controls (102 in total) to identify new targets to use in treatment of breast cancer.

Role of the tumor suppressor folliculin in breast cancer

Authors: Dex-Ann Brown-Grant*, Adaobi Mofunanya**, Ken-Ichi Takemaru* and Feng-Qian Li*
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Birt-Hogg-Dubé (BHD) syndrome, caused by germline mutations in folliculin (FLCN), is a genetic disorder with increased susceptibility to kidney cancer, renal and pulmonary cysts, and fibrofolliculomas. The human FLCN protein consists of 579 aa with no significant homology to known proteins. Roughly 88% of BHD patients carry germline mutations including insertions, deletions, nonsense mutations or splice site mutations in the coding region, which truncate the C-terminal region of the FLCN protein. To date, 55 unique germline mutations have been reported, and more than half of the patients harbor insertion/deletion mutations in a hypermutable poly$(C)_8$ tract in exon 11 of the gene. Somatic "second hit" mutations that truncate the FLCN protein or cause a loss of heterozygosity have been identified in 70% of renal tumors, suggesting a tumor suppressor role for FLCN. This was subsequently validated using various cell lines and animal models. FLCN is evolutionarily conserved across species including *C. elegans* and *D. melanogaster,* highlighting its fundamental cellular functions. More recently, the association of BHD syndrome with breast cancer has been reported, but the role of FLCN in breast tumorigenesis remains unknown. To investigate if FLCN functions as a tumor suppressor in the breast tissue, we analyzed expression levels of FLCN in a panel of human mammary carcinoma cell lines. Intriguingly, we found that FLCN protein levels were significantly decreased in MDA-MB-231, BT-474, and SK-BR-3 whereas it is down-regulated to a lesser extent in MCF-7 and T-47D cell lines, in comparison to the non-tumorigenic human mammary cell lines MCF-10A and MCF-12F. Currently, we are in the midst assessing whether FLCN expression is altered in human breast tumor samples using breast cancer cDNA arrays. In addition, we found that FLCN specifically binds to the oncoprotein β-catenin. Furthermore, we discovered that FLCN bears a RING finger-like E3 ubiquitin ligase motif and destabilizes β-catenin, and thereby regulating β-catenin signaling activity. Strikingly, aberrant activation of Wnt/β-catenin signaling has been reported in about 60% of breast cancer patients. Thus, we hypothesize that FLCN acts as a tumor suppressor in the breast tissue by serving as an E3 ubiquitin ligase to regulate β-catenin stability via proteasomal degradation. Our research will contribute to the understanding of breast tumorigenesis in BHD patients and molecular functions of FLCN as a tumor suppressor. This work is supported by the 2016-2017 TRO, Carol M. Baldwin Breast Cancer Research Award

Targeting Dihydroceramide Metabolism Reactivates Anoikis in HER2 Positive Breast Cancer

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Resistance to anoikis – cell death following detachment from the extracellular matrix (ECM) – plays a central role in metastasis, enabling cancer cells to survive in the bloodstream and foreign tissue environments. Consequently, a better understanding of the mechanisms by which oncogenes promote anoikis resistance could define new druggable targets for treating metastatic tumors, particularly important for aggressive subtypes such as HER2+ and basal breast cancer (BC). Sphingolipids (SL) are well-established mediators of cell death and SL metabolism is dysregulated in BC, yet connections between reprogramming of SL metabolism and anoikis resistance have not been investigated. In our studies, we found accumulation of pro-death SLs ceramide, dihydroceramide (dhCer) and sphingosine in normal MCF10A breast epithelial cells undergoing anoikis but not HER2+ BC cells that are anoikis resistant. Transformation of MCF10A cells with oncogenic HER2 suppressed dhCer levels, while inhibiting HER2 in BC cells increased dhCer levels in ECM-detached conditions indicating direct regulation of dhCer by HER2. Importantly, pharmacological inhibitors of DEGS1 – the primary dhCer metabolizing enzyme - reactivated anoikis in HER2+ BC cells, which resulted in decreased colony formation in soft agar. Finally, analysis of public datasets linked high levels of DEGS1 to worse relapse-free survival. Taken together, these results begin to connect HER2 reprograming of SL levels – specifically, increased dhCer metabolism – to anoikis resistance, and suggest that DEGS1 could be a novel target for metastatic tumors through reactivating anoikis pathways. They also suggest that DEGS1 expression may be a marker of metastasis prone HER2+ BC.

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