UNIVERSITY OF Cincinnati

Cincinnati Cancer Symposium Series

2016 Jensen Symposium on Breast Cancer

November 3 – 4, 2016

Hosted by Department of Cancer Biology

University of Cincinnati
College of Medicine
Cincinnati, Ohio USA

SYMPOSIUM ORGANIZERS

Sohaib Khan, Professor and Jensen Symposium Chair
Department of Cancer Biology
University of Cincinnati College of Medicine

Jun-Lin Guan, Professor and Chair of the
Department of Cancer Biology
University of Cincinnati College of Medicine

Harinder Singh, Professor
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Susan Waltz, Professor
Department of Cancer Biology
University of Cincinnati College of Medicine

Xiaoting Zhang, Associate Professor
Department of Cancer Biology
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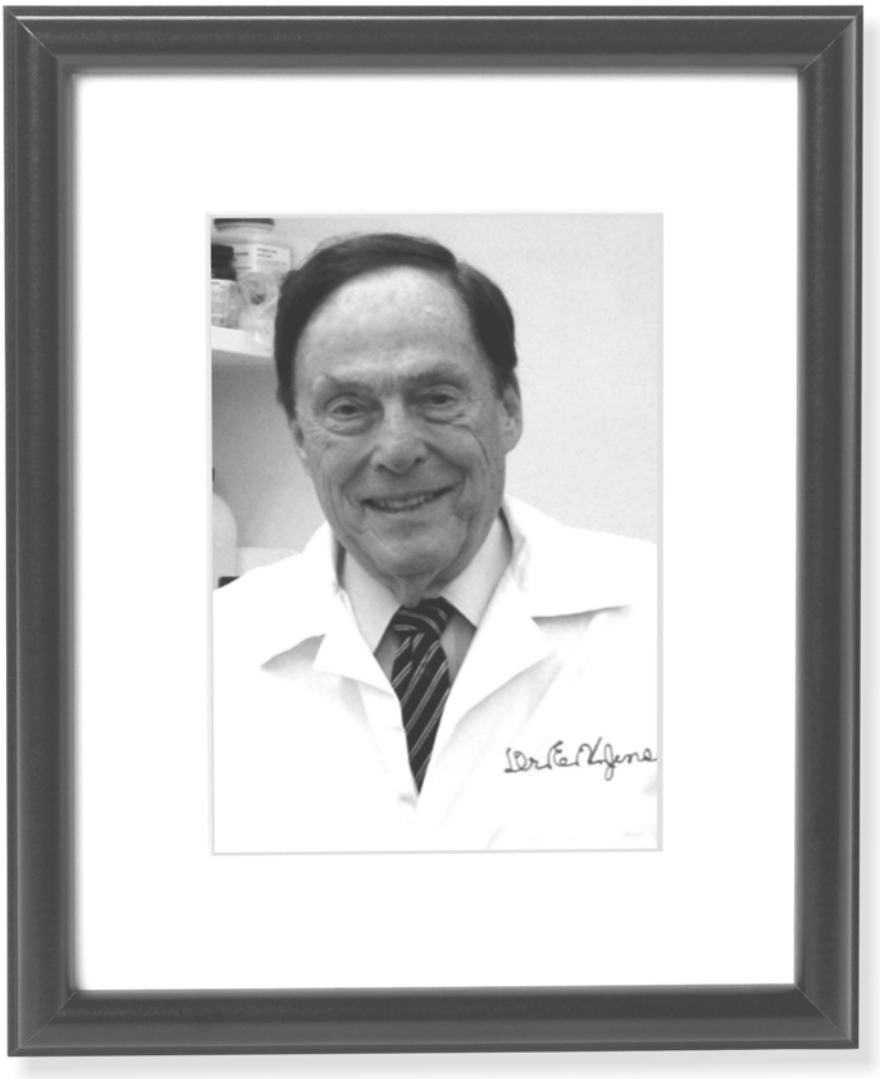

Dr. Elwood Jensen served as the University of Cincinnati Distinguished University Professor from 2002 - 2012. He gifted his collection to UC College of Medicine. Please visit the exhibit on Elwood Jensen at the Henry R. Winkler Center for the History of the Health Professions. The exhibit contains many items from the Elwood V. Jensen, Ph.D. Papers, such as the Lasker Award and other commendations, photographs, professional and personal correspondence, and much more. The displays are located in the Winkler Center on the R-Level of the Donald C. Harrison Health Sciences Library. From the library stairwell take a LEFT and walk straight ahead. If using the library elevator, take a left upon exiting, then another left and proceed to the glass doors. The Winkler Center will be open between 8 AM and 5 PM on both November 3rd and 4th.

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LOCATION**

7:30 – 5:00 Registration and Refreshments — CARE/CRAWLEY

8:00 – 8:15AM **WELCOME & OPENING REMARKS** — KRESGE AUDITORIUM

William Ball, Senior Vice President for Health Affairs and Dean
University of Cincinnati College of Medicine

Jun-Lin Guan, Chair and Professor, Department of Cancer Biology
University of Cincinnati College of Medicine

8:15 – 10:15 **SESSION 1*: Hormonal Signaling** — KRESGE AUDITORIUM

Session Chair: Sohaib Khan

Bert W. O'Malley, Baylor
Title: Structural and Functional Impacts of ER Coactivator Sequential Recruitment

Geoffrey Green, U Chicago
Title: ER and PR As Targets for Breast Cancer Treatment and Prevention

Myles Brown, Dana-Farber
Title: Hacking The Hormone Code

Short Talk 1, **Charles Foulds**, Baylor College of Medicine
Title: Proteomic Profiling Identifies Key Coactivators Utilized by Mutant ERalpha Proteins to Promote Ligand-Independent Transcription and Cell Growth

Short Talk 2, **Zeynep Madak-Erdogan**, UIUC
Title: Integrative –omics Approach Identifies Novel Roles for Extra-nuclear ERa Signaling in Rewiring Cancer Cell Metabolism During Obesity-associated Postmenopausal Breast Cancer

10:15 – 10:45 Refreshment Break / Group Photo — OUTSIDE KRESGE AUDITORIUM

10:45 – 12:30 **SESSION 2: Cancer Genome and Stem Cells** — KRESGE AUDITORIUM

Session Chair: Susan Waltz

Max Wicha, U Michigan
Title: Targeting Breast Cancer Stem Cells

Jun-Lin Guan, U Cincinnati
Title: Regulation of Breast Cancer Stem Cells By Kinase Signaling and Autophagy

Elaine Mardis, Wash U
Title: Translating The Cancer Genome

Short Talk 3, **Sandy Goyette**, University of Missouri-Columbia
Title: Progestins Induce Stem-Cell Like Properties in Hormone-dependent Human Breast Cancer Cells

		Location**
12:30 – 2:30	Lunch and Poster Session	**CARE/Crawley**
2:30 – 5:00	**SESSION 3: Tumor Microenvironment**	**Kresge Auditorium**

Session Chair: Xiaoting Zhang

Yibin Kang, Princeton
Title: Jagged1-dependent Stromal Niche Mediates Bone Metastasis and Chemoresistance of Breast Cancer

Morag Park, McGill
Title: Heterogeneity In Triple Negative Breast Cancer Microenvironment: Impact On Outcome

Susan Waltz, U Cincinnati
Title: Coordinated Signaling In The Breast Cancer Microenvironment

Zena Werb, UCSF
Title: Understanding Cancer, Metastasis and The Tumor Microenvironment In The Age of Single-Cell Genomics

Short Talk 4, **Erik Nelson**, University of Illinois at Urbana-Champaign
Title: 27-hydroxycholesterol an ER and LXR Modulator Increases Breast Cancer Metastasis Through Its Actions On the Host

Short Talk 5, **Neeraja Sathyamoorthy**, NCI
Title: Successful Grant Writing For Young Investigators

7:00 PM	**Banquet**	**Kingsgate Marriot – Grand Ballroom**

*Plenary talks: 25 min talks/5 min question period; Short Talks: 10 min talks/5 min question period

**Kresge Auditorium is located in the Medical Sciences Building

LOCATION**

7:30 – 8:00AM Refreshments — CARE/CRAWLEY

8:00 – 10:00 **SESSION 4: Gene/Transcriptional Regulation** — KRESGE AUDITORIUM

Session Chair: Shuk-Mei Ho

Robert Roeder, Rockefeller
Title: Mechanistic Studies of the Cooperative Functions of Transcriptional Co-activators

David Spector, CSHL
Title: A Nucleic Acid Target and A Nucleic Acid Drug: Interfering With Breast Cancer Progression

Xiaoting Zhang, U Cincinnati
Title: Estrogen Receptor Coactivator MED1 In Breast Cancer and As A Therapeutic Target

Short Talk 6, **Gokul Das**, Roswell Park Cancer Institute
Title: Pro-versus Anti-tumorigenic Role of Estrogen Receptor-beta in Breast Cancer is Determined by p53 Status

Short Talk 7, **Safiya Khurshid**, Ohio State University
Title: Suppression of Anti Tumor immunity by E2F3

10:00 – 10:30 Refreshment Break — CARE/CRAWLEY

10:30 – 12:00 **SESSION 5: Cancer Targeting** — KRESGE AUDITORIUM

Session Chair: Elyse Lower

Carlos Arteaga, Vanderbilt
Title: Presurgical Trials With Antiestrogens For Discovery of Mechanisms of Endocrine Resistance

Donald P. McDonnell, Duke
Title: Identification of Targetable Proteins and Processes Downstream of Steroid Receptors in Breast and Prostate Cancers

Short Talk 8, **Tom Cunningham**, University of Cincinnati
Title: A Re-wired Metabolic Circuitry Fuels Cancer Development: Implications for Diagnosis and Targeted Therapy

Short Talk 9, **Michael Wendt**, Purdue University
Title: Epithelial-mesenchymal Plasticity Drives Growth Factor Discordance in Metastatic Breast Cancer

12:00 – 1:00 Lunch — CARE/CRAWLEY

1:00 – 3:30 **SESSION 6: Tumor Dormancy** — KRESGE AUDITORIUM

Session Chair: Harinder Singh

LOCATION**

Jeff Rosen, Baylor
Title: The Role of The Tumor Microenvironment In Tumor Dormancy, Recurrence and Metastasis

Lewis Chodosh, U Penn
Title: Breast Cancer Recurrence: When The Dragon Awakes

Shuk-Mei Ho, U Cincinnati
Title: Estrogen Receptor ß5 Inhibits Bcl2L12 Function and Confers Better Breast Cancer Survival

Craig Jordan, MD Anderson
Title: Models and Mechanisms of Acquired Resistance To Antiestrogenic Treatment Strategies

Short Talk 10, **Kenneth P. Nephew**, Indiana University Melvin and Bren Simon Cancer Center
Title: Impact of Ethnicity-dependent Differences in Normal and Tumor Epigenome on Breast Cancer Progression

Short Talk 11, **James L. Wittliff**, U Louisville
Title: A Rosetta Stone for Deciphering Breast Cancer Genomics to Identify Molecular Targets & Diagnostics

3:30 – 3:35PM Concluding Remarks — KRESGE AUDITORIUM

*Plenary talks: 25 min talks/5 min question period; Short Talks: 10 min talks/5 min question period

**KRESGE AUDITORIUM is located in the Medical Sciences Building

SESSION 1: Hormonal Signaling **Thursday, November 3**

Structural and Functional Impacts of ER Coactivator Sequential Recruitment

Bert W. O'Malley,

Ping Yi, Zhao Wang, Qin Feng, Michael F. Schmid, Mien-Chie Hung, Wah Chiu

Baylor College of Medicine, Houston, TX

Estrogen receptor (ER) is a transcription factor critical for development, reproduction, metabolism and cancer. ER function hinges on its ability to recruit primary and secondary coactivators. Portions of ER bound to small domains of coactivator proteins have been studied structurally, but it is unclear how intact ER and coactivators are assembled into a transcriptionally active complex on DNA. We used cryo-EM to determine the quaternary structure of an active complex of DNA-bound ERalpha steroid receptor coactivator 3 (SRC-3) and a secondary coactivator (p300). Each of the two ligand-occupied ER monomers recruits one SRC-3 molecule via its transactivation function domain; the two SRC-3s in turn bind one molecule of p300 through multiple contacts. The arrangement of these components can explain how p300 is accessible to nearby histones without steric hindrance from the other proteins in the complex. Nuclear receptors recruit multiple coactivators sequentially to activate transcription. Recently, we uncovered the process of "ordered" recruitment whereby different coactivator activities engage the nuclear receptor complex at different steps of transcription. For example, after ER recruits SRC-3 and p300/CBP, the dynamic complex changes by recruitment of CARM1. CARM1 recruitment lags behind the binding of SRC-3 and p300 to ER. Again combining cryo-EM structure analysis and biochemical approaches, we demonstrate that there is a close crosstalk between early and late recruited coactivators. The sequential recruitment of CARM1 not only adds a protein arginine methyltransferase activity to the ER-coactivator complex but also alters the structural organization of the pre-existing ER/SRC-3/p300 complex. It induces a p300 conformational change and significantly increases p300 HAT activity on histone H3K18 residues, which in turn promotes CARM1 methylation activity on H3R17 residues to enhance activation of transcription. The N-terminal domain of CARM1 is indispensable for the interaction with p300 and contains an ability to alter the function of ER/SRC-3/p300 complex. Taken together, our studies reveal a structural role for coactivator sequential recruitment and further highlight the biochemical process that mediates ER activation of transcription.

SESSION 1: Hormonal Signaling **Thursday, November 3**

ER and PR as Targets for Breast Cancer Treatment and Prevention

Geoffrey Greene

University of Chicago, Chicago, IL

Estrogen receptor alpha (ER) and progesterone receptor (PR) are widely used predictive and prognostic biomarkers in breast cancer. ER is also a well-established therapeutic target. A major unresolved clinical issue is the development of therapy resistance, especially to ER-targeted therapies. We, and others, have observed somatic ESR1 mutations in 20-25% of metastatic tumors obtained from women who have acquired resistance to endocrine therapies. The two most common mutations are Y537S and D538G, both of which stabilize and/or facilitate the formation of an active AF-2 conformation in the ER LBD. A combination of structural, biophysical, cell and animal studies have helped define the underlying molecular mechanisms that account for AI/SERM/SERD resistance, which is already contributing to the development of SERMs and/or SERDs with improved clinical utility.The clinical value of PR remains controversial and the role of PR is poorly understood. We have recently observed that PR reprograms and modulates estrogen signaling. Importantly, PR functions as a genomic ER agonist, while acting as a phenotypic antagonist in ER+/PR+ breast cancer models. Animal studies of ER+/PR+ T47D human breast tumor xenografts to jointly targeted therapies demonstrate that a combination of tamoxifen and certain selective progesterone receptor modulators (SPRMs) synergistically inhibits tumor explant growth and promotes tumor regression in athymic mice compared to either treatment alone. Our results indicate that PR is an essential modulator of ER action and that appropriate co-targeting of ER and PR should be exploited clinically.

SESSION 1: Hormonal Signaling **Thursday, November 3**

Hacking the Hormone Code

Myles Brown

Dana-Farber Cancer Institute, Boston, MA

Endocrine therapies targeting the estrogen receptor (ER) are the mainstay of treatment for the majority of breast cancers. While ER antagonists and aromatase inhibitors are effective adjuvant therapy for patients with early stage ER+ breast cancers, patients with advanced disease invariably develop resistance. Using genome-wide CRISPR screens we have identified genes essential for the growth of ER+ breast cancers including key components of the ER signaling pathway such as ER itself, ER coregulators and transcription factors we previously identified as playing important roles in ER function. Importantly, we also identified genes whose loss confers endocrine resistance. Most strikingly, loss of CSK, a negative regulator of SRC family kinases (SFK), is sufficient to drive estrogen independent growth in culture and in xenografts and to confer resistance to ER antagonists. Examination of the ER cistrome revealed an ER bound enhancer upstream of the CSK transcription start site and deletion of this enhancer was also sufficient to block the estrogen induction of CSK and to promote estrogen independent growth. A synthetic lethality screen for genes essential in the absence of CSK identified the PAK2 kinase. Inhibitors of PAK2 or SFK block estrogen-independent breast tumor growth and synergize with the complete ER antagonist fulvestrant. These findings reveal an estrogen-induced negative feedback loop that constrains the growth of ER+ tumors thereby limiting the efficacy of therapies that inhibit ER and suggest a previously unappreciated therapeutic route to overcoming endocrine resistance.

SHORT TALK #1: **Thursday, November 3**

Proteomic Profiling Identifies Key Coactivators Utilized by Mutant ERalpha Proteins to Promote Ligand-Independent Transcription and Cell Growth

Charles Foulds

Baylor College of Medicine, Houston, TX

Approximately 70% of breast cancers are estrogen receptor alpha (ER-alpha)-positive and are driven by estrogen. Targeting ER-alpha with endocrine therapies provides effective adjuvant treatment with reductions in patient recurrence approaching 50%. Still, many tumors acquire resistance, and in ~20% of these cases, gain-of-function mutations in the ER-alpha gene (ESR1) are implicated. Amino acid substitutions in the receptor's ligand binding domain (LBD) result in receptors displaying ligand-independent activity and endocrine therapy resistance. In addition, rarer translocations of ESR1 to other genes create fusion proteins that lack the LBD and cannot be targeted with anti-estrogens. Our studies focus on the most prominent of the ER-alpha LBD mutants, Y537S and D538G, and the ESR1-YAP1 fusion protein. We propose that therapeutic strategies designed to target coactivators (CoAs) binding a mutant receptor offer a unique approach to enhance treatment of recurrent disease. To define the set of CoAs binding a particular mutant receptor, a mass spectrometric (MS) approach to profile the CoA "complexome" for each mutant ER-alpha protein was performed. MS data of CoA recruitment to each mutant was compared to the wild-type (WT) receptor to identify potential new targets. We observed specific CoAs displaying enhanced binding to each mutant ER-alpha receptor. Importantly, inhibiting these CoA candidates (with small molecule inhibitors and/or siRNA) reduced the ability of the different ERa mutants to activate ERE-driven gene transcription and to promote breast cancer cell growth. Thus, inhibition of distinct CoAs may be a promising new therapeutic strategy to inhibit growth of ER-alpha-mutant expressing breast cancers.

SHORT TALK #2: **Thursday, November 3**

Integrative –omics Approach Identifies Novel Roles for Extra-nuclear ERa Signaling in Rewiring Cancer Cell Metabolism During Obesity-associated Postmenopausal Breast Cancer

Zeynep Madak-Erdogan,

Zhao, Yiru; Rosso, Gianluigi; Kulkoyluoglu-Cotul, Eylem; Kim, Sung Hoon; Katzenellenbogen, John; Flaws, Jodi; Smith, Rebecca

University of Illinois at Urbana-Champaign, Urbana, IL

Obesity is a preventable risk factor for post-menopausal ERa(+) breast cancer. We hypothesized that serum from obese post-menopausal women contain factors that would increase tumorigenicity of breast cancer cells. Using whole metabolite profiling and OLINK biomarker panel of about 400 proteins associated with cancer, inflammation and cardiovascular disease, we identified biomarkers that were differentially present in serum from 100 obese and non-obese postmenopausal women. Next, using in vitro cell based assays as proxy we identified free fatty acids (FFAs) as factors from serum that correlate with increased cell proliferation, motility and mTOR activation in ERa(+) breast cancer cells. We performed RNA-Seq, ERa ChIP-Seq and metabolomics analysis in breast cancer cells that are exposed to conditions that mimic serum from obese postmenopausal women. This integrative –omics approach enabled us to uncover ERa and mTOR pathway-dependent metabolic rewiring in breast cancer cells under these conditions. Pathway preferential estrogens (PaPEs), which target ERa and mTOR signaling, were able to block free fatty acid-dependent proliferation of breast cancer cells. In fact, efficient cancer cell killing by PaPEs was achieved only in the presence of FFAs, suggesting a role for obesity-associated metabolic rewiring in providing new vulnerabilities for the breast cancer cells. In summary, we uncovered a novel role for extranuclear-initiated ERa signaling in rewiring breast cancer cell metabolism in response to obesity-associated factors in the serum. Our findings provide a basis for preventing or inhibiting obesity-associated breast cancer by using PaPEs that would exploit new metabolic vulnerabilities of breast tumors in obese postmenopausal women

SESSION 2: Cancer Genome and Stem Cells **Thursday, November 3**

Targeting Breast Cancer Stem Cells

Max Wicha

University of Michigan Comprehensive Cancer Center, Ann Arbor, MI

SESSION 2: Cancer Genome and Stem Cells **Thursday, November 3**

Regulation of Breast Cancer Stem Cells by Kinase Signaling and Autophagy

Jun-Lin Guan

Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH

Cancer stem cells (CSCs) are intimately involved in the re-growth of metastasized tumors in distant organs, local recurrence of the disease as well as resistance to various chemotherapeutic agents and radiation therapy. They are the lethal seeds of both the primary tumor and more importantly metastasis, and their numbers and activity are regulated by both intrinsic tumor cell functions such as autophagy and metabolic activity, as well as tumor microenvironment. Focal adhesion kinase (FAK) has been implicated in the development of cancers including those of the breast. Consistent with its key role in integrin signaling, several groups including us showed that FAK regulates various functions in both tumor and stromal cells that contribute to cancer development, metastasis and drug resistance. Our studies using a conditional FAK mutant knockin allele showed distinct role of its kinase and scaffolding functions in the regulation breast CSC formation and maintenance in different subtypes of breast cancer. FIP200 (FAK-family Interacting Protein of 200 kDa) was initially identified as a protein inhibitor for FAK and its related kinase Pyk2, and subsequently shown to be a component of the ULK1/Atg13/FIP200/Atg101 complex essential for autophagy induction. We showed that FIP200 ablation decreased mammary tumor development, growth, and metastasis, providing the first evidence for a pro-tumorigenesis role for autophagy in animals with intact immune systems. We also found recently the co-existence of distinct BCSCs as identified by ALDH+ and CD29hiCD61+ markers, respectively, in murine models of breast cancer. While both BCSCs exhibit enhanced tumor initiating potential, CD29hiCD61+ BCSC displayed increased invasive abilities and higher expression of epithelial to mesenchymal (EMT) and mammary stem cell-associated genes, whereas ALDH+ BCSC were more closely associated with luminal progenitors. Very interestingly, FIP200 deletion diminished the tumor-initiating properties of both ALDH+ and CD29hiCD61+ BCSCs, which was achieved by impairing the Stat3 and TGFß/Smad pathways, respectively. Together, these studies provide significant insights into the mechanisms of regulation of BCSCs by various kinase signaling pathways and autophagy, which will facilitate future efforts on how to tailor drug combinations to improve therapeutic efficacy.

SESSION 2: Cancer Genome and Stem Cells

Translating the Cancer Genome

Elaine R. Mardis

McDonnell Genome Institute at Washington University School of Medicine, St. Louis MO

Large-scale cancer genomics discovery has yielded remarkable insights to our understanding of how changes in DNA result in the onset and progression of cancer. Certainly, the decreasing cost of massively parallel sequencing has featured prominently in the numbers and types of samples that can be studied, as has the remarkable methods and computational analysis methods that have been devised over the past eight years. Our field is now poised to transition this basic knowledge about cancer genomics to benefit cancer patients, in a number of ways. I will present several vignettes from our translational research efforts to illustrate the various components needed to make this transition effectively; advanced methods that address the vagaries of clinical samples, analytical approaches that provide an integrated and highly specialized set of results for clinical use, and cancer-specific, curated databases that are necessary to help organize, communicate and keep current our increasing body of knowledge about cancer genomics.

SHORT TALK #3: **Thursday, November 3**

Progestins Induce Stem-Cell Like Properties in Hormone-dependent Human Breast Cancer Cells

Sandy Goyette, Yayun Liang, Moiz Munir and Salman M Hyder

Department of Biomedical Sciences and Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO

Clinical trials show that post-menopausal women undergoing combined hormone replacement therapy containing estrogen (E) and progestins (P) have an increased risk of breast cancer compared with women taking E alone. We showed that both natural and synthetic P accelerate the development of breast tumors *in vivo* and increase lymph node metastasis, leading us to hypothesize that P-induced tumor growth and metastasis may be mediated by an enrichment of the cancer stem cell (CSC) pool. With this in mind we used flow cytometry to examine whether P influences the expression of CSC markers (CD44 and ALDH) in breast cancer cells and found that natural P, as well as a variety of synthetic P (10 nM) increased CD44 levels in both T47-D and BT-474 cells. Induction of CD44 was blocked by the antiprogestin RU-486, demonstrating that induction of CD44 is progesterone receptor dependent. Other steroid hormones had no effect on CD44 expression. A subset of the induced $CD44^{high}$ cells demonstrated high ALDH enzyme activity. The synthetic progestins medroxyprogesterone acetate and norethindrone significantly increased mammosphere formation, suggesting that enrichment of the $CD44^{high}$ and $ALDH^{bright}$ subpopulation of cancer cells is of functional significance. Based on our observations we contend that exposure of breast cancer cells to synthetic P may lead to an enrichment of the CSC-like pool, supporting the development of P-accelerated tumors *in vivo*. Supported by generous gifts from donors of Ellis Fischel Cancer Center, and by a Faculty award from the College of Veterinary Medicine, University of Missouri, Columbia.

SESSION 3: Tumor Microenvironment **Thursday, November 3**

Jagged1-dependent Stromal Niche Mediates Bone Metastasis and Chemoresistance of Breast Cancer

Yibin Kang

Department of Molecular Biology, Princeton University, Princeton, NJ

Bone metastasis is a frequent occurrence in breast cancer, affecting more than 70% of late stage cancer patients with severe complications such as fracture, bone pain, and hypercalcemia. The pathogenesis of osteolyitc bone metastasis depends on cross-communications between tumor cells and various stromal cells residing in the bone microenvironment. Several growth factor signaling pathways, secreted miRNAs and exosomes are functional mediators of tumor-stromal interactions in bone metastasis. We showed that TGFß is released from bone matrix upon bone destruction, and signals to breast cancer to further enhance their malignancy in developing bone metastasis. We furthered identified Jagged1 as a TGFß target genes in tumor cells that engaged bone stromal cells through the activation of Notch signaling to provide a positive feedback to promote tumor growth and to activate osteoclast differentiation. Using genetically modified mouse models, we revealed a surprising role of Jagged1 in promoting primary tumor growth and chemoresistance, suggesting diverse functions of the Jagged1 in multiple-stage of tumor progression by engaging different stromal cells encountered in the microenvironment. Chemotherapy of bone metastasis induced elevated expression of Jagged1 in osteoblasts, which provide a pro-survival niche for tumor cells in the bone. These findings support the notion that organ-specific metastatic traits can be developed in the primary tumor microenvironment as tumor cells adapt to the selective pressure of the microenvironment. Importantly, therapeutic targeting of Jagged1 using a humanized monoclonal antibody reduce primary tumor growth, bone metastasis, and sensitize tumors to chemotherapy.

SESSION 3: Tumor Microenvironment **Thursday, November 3**

Deconvolution of the Triple-Negative Breast Cancer Microenvironment

Morag Park

Tina Gruosso[1], Mathieu Gigoux[1], Sadiq Saleh[1], Nicholas Bertos[1], Atilla Omeroglu[2], Dongmei Zuo[1], Sarkis Meterissian[4], Michael Hallett[5]

Goodman Cancer Research Center, [1]Dept. of Biochemistry, [2]Dept. of Pathology, [3]Dept. of Surgery, [4]McGill Centre for Bioinformatics, McGill University, Montreal, Canada.

Breast cancer heterogeneity is one of the principal obstacles both to predicting outcome and to determining an effective course of treatment for this disease. Although genomic technologies have been used to gain a better understanding, by identifying gene expression signatures associated with clinical outcome and breast cancer subtypes, relatively little is known about heterogeneity in the tumor microenvironment. It is now accepted that changes in the normal cells that constitute the tumor microenvironment (TME) play important roles in determining cancer progression and ultimate outcome. We and others have established that an immune gene expression signature correlates with good outcome in triple negative breast cancer (TNBC), yet fails to accurately predict outcome in all patients. Examining stromal heterogeneity in TNBC has identified four distinct stromal clusters, three of which are prognostic, contain distinct immune signatures and a signature of fibrosis. Lymphocytic infiltration and access to tumor parenchyma is not well understood due to high levels of spatial heterogeneity within tumors. We show that location of CD8+T cells is strongly influenced by TME subtypes and identify gene expression signatures predictive of distinct CD8+T cell localization and patient outcome. Since mounting evidence(suggests that immune-checkpoint inhibitor immunotherapies may be promising for(only a subset of TNBC patients, highlights the importance of understanding how the TME influences CD8+T cell location.

SESSION 3: Tumor Microenvironment **Thursday, November 3**

Coordinated Signaling In The Breast Cancer Microenvironment

Susan E. Waltz

Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH

Breast cancer (BC) is the most common cancer amongst women in the US with an estimated 232,670 new cases of BC expected to be diagnosed this year. While treatment advances and earlier detection have contributed to a decline in BC death rates, 40,000 women and 430 men in the US are still expected to die from BC this year. These sobering mortality figures are a consequence of our lack of effective preventive measures and treatments. Thus, understanding novel mechanisms driving BC and defining new treatment strategies that have the ability to combat the growth and spread of this disease are urgently needed.

Our laboratory is focused on examination of the Ron receptor tyrosine kinase and its ligand, hepatocyte growth factor-like protein (HGFL) in breast pathophysiology. Ron is highly expressed in about 50% of human breast cancers (BCs), with overexpression strongly correlating with poor prognosis, metastasis and death in human patients. Recent data have also established that HGFL is upregulated in human and murine BCs and functions to promote oncogenic Ron activation and BC metastasis. Studies from our laboratory utilizing mice with a genetic loss of HGFL or Ron in murine BC models have shown that HGFL and Ron expression support a permissive tumor microenvironment that allows for and drives aggressive BC tumor growth and metastasis. Further, in studies using mice with a conditional loss of Ron signaling in either macrophages or breast epithelial cells, we show that Ron expression in either cell type is required for efficient tumorigenesis. In total, our data suggest a complex interplay exists between Ron expressing cell types in the tumor microenvironment which is responsible for the aggressive phenotype observed in Ron expressing BCs. Current studies are underway and will be aimed at examining the consequences and mechanisms behind the coordinated activation of this signaling cascade in breast tumorigenesis.

SESSION 3: Tumor Microenvironment **Thursday, November 3**

Understanding Cancer, Metastasis and The Tumor Microenvironment In The Age of Single-Cell Genomics

Zena Werb

University of California, San Francisco, CA

Department of Anatomy and the Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, California

Metastasis is the major cause of poor outcome in breast cancer. The cancer cells within tumors are heterogeneous and can have properties as divergent as self-renewal, tumor initiation and repopulation potential, dormancy, evasion of cell death and metastasis. The cancer initiating cells are believed to reside in niches where proliferation and differentiation are. We have exploited patient-derived xenograft (PDX) models of human breast cancer to analyze metastatic cells in peripheral tissues. We used microfluidic PCR and RNA-seq analysis to evaluate cancer development and metastasis at the single cell level and found that the inflammatory microenvironment of metastatic sites was distinct from that of the parental tumor and the normal tissue, even before metastasis took hold. The metastatic cells from tissues with a low metastatic burden showed increased expression of stem cell, pro-survival, and dormancy-associated genes. In contrast, in macrometastases, the cells were more similar to primary tumor cells with higher proliferative genes. Understanding the processes involved in metastasis is critical for developing new preventative and therapeutic strategies. Funded by the National Cancer Institute.

Lawson, D. A., N. Bhakta, K. Kessenbrock, K. Prummel, Y. Yu, K. Takai, A. Zhou, H. Eyob, S. Balakrishnan, C.-Y. Wang, P. Yaswen, A. Goga & Z. Werb (2015). Single-cell analysis reveals a stem cell program in early human metastatic breast cancer cells. Nature. 526:131–135.

Plaks, V., N. Kong & Z. Werb (2015). The cancer stem cell niche: How essential is the niche in regulating stemness of tumor cells? Cell Stem Cell 16: 225-238.

SHORT TALK #4: **Thursday, November 3**

27-hydroxycholesterol an ER and LXR Modulator Increases Breast Cancer Metastasis Through Its Actions on the Host

Erik Nelson

University of Illinois at Urbana-Champaign, Champaign, IL

Breast cancer remains the second leading cause of cancer death in women. Associated mortality is most often due to the metastatic spread of breast cancer, highlighting the need for novel approaches to prevent and treat this stage of disease. In this regard, both obesity and elevated cholesterol have been associated with a decreased relapse free survival time. Furthermore, patients taking inhibitors of HMGCoA-reductase (HMGCR, statins) experience a significantly increased relapse free survival time. Therefore, we tested the hypothesis that cholesterol promotes the metastasis of breast cancer. Since we have shown that a primary metabolite of cholesterol, 27-Hydroxycholesterol (27HC), exerts partial agonist activity on both the estrogen receptors (ERs) and liver X receptors (LXRs), we determined whether 27HC may mediate the potential metastatic effects of cholesterol. Interestingly, we demonstrate that 27HC does increase metastasis to the lung, but its activities require the presence of host immune cells. Indeed, we find that 27HC engages immune cells to alter the metastatic microenvironment in such a way that facilitates cancer cell colonization and growth. Our ongoing work is aimed at identifying the specific mechanisms by which 27HC induces these changes and thereby increases metastasis. In summary, our data strongly suggests that 27HC is the biochemical mediator of the effects of cholesterol on breast cancer metastasis, providing additional support for the exploration of lower cholesterol diets, pharmacological inhibitors of either HMGCR or the enzyme responsible for the synthesis of 27HC (CYP27A1), and immune cell targeting strategies in the treatment of metastatic breast cancer.

SHORT TALK #5: **Thursday, November 3**

Successful Grant Writing For Young Investigators

Neeraja Sathyamoorthy

NCI-National Cancer Institute, Bethesda, MD

SESSION 4: Gene/Transcriptional Regulation **Friday, November 4**

Mechanistic Studies of the Cooperative Functions of Transcriptional Co-activators

Robert G. Roeder,

Shu-Ping Wang, Zhanyun Tang, Tomoyoshi Nakadai and Miho Shimada

Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY

Transcriptional regulation by gene- and cell-specific DNA-binding factors underlies key events in development and in cell growth, differentiation and transformation. However, their effects on specific genes depend upon complex arrays of cofactors (co-activators and co-repressors) whose biochemical mechanisms are not completely understood. These cofactors include both chromatin remodeling/histone modifying factors (e.g., the p300/CBP histone acetyl-transferases and the SET1/MLL H3K4 methyl-transferases) and other factors (e.g., Mediator) that facilitate more direct communication between promoter-bound regulatory factors and the general transcription machinery. Emphasizing biochemical studies with cell-free systems reconstituted with recombinant chromatin templates and purified transcription factors, the cooperative functions and mechanism of action of selected co-activators will be discussed in relation to gene regulation by selected activators (including nuclear hormone receptors). This may include recent studies of p300-dependent activation of chromatin templates through novel acylation marks as well as mechanisms for the de-compaction and activation of higher order (linker histone H1-containing) chromatin templates.

SESSION 4: Gene/Transcriptional Regulation **Friday, November 4**

A Nucleic Acid Target and a Nucleic Acid Drug: Interfering with Breast Cancer Progression

Spector, David

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Genome-wide analyses have identified thousands of long non-coding RNAs (lncRNAs). With breast cancer being the most frequent malignancy in women worldwide, we set out to investigate the potential of lncRNAs as novel therapeutic targets. Malat1 is among the most abundant lncRNA whose expression is altered in numerous cancers. We have found that genetic loss, or systemic knockdown, of Malat1 using antisense oligonucleotides, in the MMTV-PyMT mouse mammary carcinoma model results in slower tumor growth accompanied by differentiation into cystic tumors and a significant reduction in metastasis. Further, Malat1 loss results in a reduction of branching morphogenesis in MMTV-PyMT and Her2/neu amplified tumor organoids, increased cell adhesion and loss of migration. At the molecular level, Malat1 knockdown results in alterations in gene expression and changes in splicing patterns of genes involved in differentiation and pro-tumorigenic signaling pathways. Together, these data demonstrate a functional role of Malat1 in regulating critical processes in mammary cancer pathogenesis. Thus, Malat1 represents an exciting therapeutic target and Malat1 ASOs a therapeutic for inhibiting breast cancer progression.

SESSION 4: Gene/Transcriptional Regulation **Friday, November 4**

Estrogen Receptor Coactivator MED1 in Breast Cancer and As a Therapeutic Target

Xiaoting Zhang

Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH

Recent studies have established Mediator Subunit 1 (MED1) as a key ER transcriptional coactivator in both normal mammary gland development and breast cancer. Significantly, the MED1 gene is located at the chromosome 17q12 region, also known as the HER2 amplicon, and co-amplifies with HER2 in almost all instances. Importantly, we found that MED1 serves as a key crosstalk point for the HER2 and ERa pathways in anti-estrogen resistance of breast cancer. We have now generated MED1 mammary specific overexpression mice and crossed with MMTV-HER2 mice. Significantly, we observed greatly accelerated tumor onset, growth, multiplicity, and tumor metastasis in MMTV-HER2/MMTV-MED1 double transgenic mice. Our further in vitro and in vivo studies revealed critical roles and underlying molecular mechanisms for MED1 in cancer stem cell formation and metastasis during HER2-mediated mammary tumorigenesis. These studies not only for the first time reported key roles for a HER2 amplicon co-amplified gene in HER2-driven tumorigenesis but also support MED1 as a potential therapeutic target. To test this, we have assembled three-way junction (3-WJ) based pRNA-HER2apt-siMED1 nanoparticles. We found these RNA nanoparticles have a very high Tm value, and are ultra-stable under various conditions including RNase A and serum treatments. Importantly, pRNA-HER2apt-siMED1 nanoparticles could specifically target HER2+ human breast cancer, efficiently deplete the expression of MED1 and decrease ER-mediated gene transcription both in vitro and in orthotopic xenograft mouse models. Most significantly, pRNA-HER2apt-siMED1 nanoparticles not only greatly reduce the growth, metastasis and mammosphere formation of HER2+ breast cancer, but also strongly sensitize them to tamoxifen treatment.

SHORT TALK #6: **Friday, November 4**

Pro-versus Anti-tumorigenic Role of Estrogen Receptor-beta in Breast Cancer is Determined by p53 Status

Gokul M. Das[1],

Utpal K. Mukhopadhyay[1], Sanjay Bansal[1], Nadi Wickramasekera[1], Rajesh Medisetty[1], Christina Adams[1], Wendy M. Swetzig[1], Austin Miller[1], Jianmin Wang[1], Chetan Oturkar[1], Alka Mukhopadhyay[1], Santhi Konduri[2]

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Whether estrogen receptor beta (ERß) is an oncogenic or a tumor suppressor protein in breast cancer has been controversial. ERß levels are high in ERa negative cancers including triple-negative breast cancer (TNBCs). Recent reports including the Cancer Genome Atlas (TCGA) show that about 80% of TNBC express mutant p53 (mut-p53) and it is the most predominant driver in these cancers. We tested the hypothesis that p53 status in breast cancer will have an important role in determining function of ERß. We report that ERß directly binds to p53 in human breast cancer cells and have mapped the domains of interaction. Using the highly sensitive proximity ligation assay (PLA), we show ERß-p53 interaction in situ in breast cancer cells and TNBC tissues expressing either wt- or mut-p53. Surprisingly, we found that ERß has opposite functions depending on the wt/mut status of p53. In the context of the wt-p53, ERß is pro-proliferative, whereas in the context of mut-p53, ERß is anti-proliferative. ERß binds and sequesters mut-p53 from mut-p53-p73 complex leading to reactivation of tumor suppressor p73. Combination of immunohistochemistry (IHC) and PLA in TNBC patient tissue microarray (TMA) followed by correlative analysis of linked database of patient tumor characteristics and disease outcome showed that p53 status is an important determinant of pro- versus anti-tumorigenic role of ERß in these tumors. These data would have significant clinical implications in targeting ERß and mutant p53 signaling pathways for diagnostic, prognostic, and therapeutic purposes especially in ERa negative cancers such as TNBC.

SHORT TALK #7: **Friday, November 4**

Suppression of Anti Tumor immunity by E2F3

Safiya Khurshid

Ohio State University, Columbus, OH

Activation of the HER2-CCND1-CDK4 pathway in breast cancer is believed to culminate in the loss of RB function, release of E2F transcriptional activity and uncontrolled tumor cell proliferation. Using conditional gene knockout strategies in mice we demonstrate that ablation of the E2f3 family member delays HER2 mammary tumorigenesis and promotes regression of established HER2-tumors. Surprisingly, the loss of E2f3 affected neither proliferation nor apoptosis of tumor cells, but rather resulted in activation of p19ARF, p53 and p53 targets such as Lif and the execution of anti-tumor immune responses. We can abrogate this delay in tumorigenesis caused by loss of E2f3 when we knockout the CD8 positive cells of the immune system. These findings shift the current paradigm of the RB-E2F axis in breast cancer beyond cell cycle control, to include oncogene-specific immune-suppressive functions.

SESSION 5: Cancer Targeting **Friday, November 4**

Presurgical Trials With Antiestrogens For Discovery of Mechanisms of Endocrine Resistance

Carlos Arteaga

Vanderbilt University, Nashville, TN

Inhibition of ER+ breast tumor cell proliferation after a short course of antiestrogen therapy correlates with long-term outcome in patients with hormone-dependent breast cancer. We applied this approach to a cohort of 155 ER+/HER2– newly diagnosed breast cancers treated with the aromatase inhibitor letrozole prior to surgical resection to identify mechanisms of endocrine resistance. The post-treatment Ki67 index revealed that a proportion of tumors remained highly proliferative and, thus, would likely harbor genes associated with intrinsic resistance to antiestrogens. To identify genomic alterations associated with resistance, tumors were subjected to whole-exome and RNA sequencing. We noted a strong correlation between amplification of 8p11-12/11q13 amplicon genes, including *WHSC1L1*, *FGFR1*, *CCND1* and *FGF3/4/19*, and a high Ki67 index, which we confirmed by fluorescence *in situ* hybridization (FISH). Targeted sequencing of serial pre-surgical, post-chemotherapy, and recurrent ER+ tumors also revealed concordant amplification of 8p11-12/11q13 genes. Overexpression of FGFR1 or cyclin D1 in ER+ MCF7 cells enhanced proliferation and estrogen independence. Additionally, combined pharmacological inhibition of FGFR and CDK4/6 reversed antiestrogen resistance in ER+ breast cancer cells harboring co-amplification of *FGFR1* and *CCND1*. From RNA sequencing, we identified a number of intrachromosomal *ESR1* fusion transcripts and gene expression signatures indicative of enhanced E2F-mediated transcription and cell cycle processes, all of which were associated with resistance. These data suggest a short course of pre-operative estrogen deprivation followed by tumor genomic profiling can be used to identify druggable alterations associated with intrinsic endocrine resistance.

SESSION 5: Cancer Targeting **Friday, November 4**

Identification of Targetable Proteins and Processes Downstream of Steroid Receptors in Breast and Prostate Cancers

Donald P. McDonnell

Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC

The focus of the research in our group continues to be the development of strategies to target estrogen and androgen signaling pathways in breast and prostate cancer. Our most recent efforts in this regard have focused on identifying proteins and processes downstream of these receptors that can be targeted in cancer. This shift in emphasis reflects the growing feeling in the field that breast and prostate cancer cells are so addicted to AR and ER that inhibition of these receptors results in the compensatory up regulation of processes that increase the activity of the receptors (i.e. up regulation of coregulators or receptors), select for gain of function receptor mutations and/or splice variants, or which increase the intratumoral production of estrogens and androgens. Given that these receptors are key components in endocrine feedback loops that modulate their activity, such compensatory responses are hardwired into these signaling pathways. This understanding has directed us to explore the feasibility of targeting proteins/processes that are downstream of these receptors and which are not in and of themselves involved in feedback signaling. It is anticipated that the activity of such targets would not be self-limited by increased activity of the receptor signaling pathways. The progress in identifying such targetable processes in breast and prostate cancer will be the focus of this presentation.

SHORT TALK #8: **Friday, November 4**

A Re-wired Metabolic Circuitry Fuels Cancer Development: Implications for Diagnosis and Targeted Therapy

Tom Cunningham

Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH

In stark contrast to normal cells that only divide a finite number of times, cancerous cells proliferate indefinitely. In order to fuel this bioenergetically and biosynthetically demanding process, oncogenic pathways hijack cellular metabolism to increase the uptake of available nutrients while simultaneously maximizing the anabolic potential of those nutrients. We have previously uncovered a novel mechanism critical for cancer development whereby oncogenic signaling coordinates the proportional increases in protein synthesis and nucleotide metabolism, via the regulation of a single rate-limiting enzyme, phosphoribosyl-pyrophosphate synthetase 2 (PRPS2). We have developed new genetic, pharmacologic, and chemical tools to exploit this specific dependency of cancer cells on PRPS2-mediated nucleotide production, thus laying the groundwork for innovative approaches to better diagnose and treat patients with cancers that are currently “undruggable”. In this regard, we have generated PRPS2 knockout mice, which exhibit no obvious phenotypic differences when compared to wild-type counterparts, yet display a dramatic reduction in tumor formation when crossed to various genetically-engineered mouse models of cancer. Additionally, in collaboration with Michael Evans at UCSF, we have designed a novel PRPS2 radiosubstrate mimetic capable of non-invasively imaging cancers in vivo. In conclusion, we have unraveled a novel feed-forward anabolic circuit that fuels cancer development upon a variety of oncogenic lesions. Moreover, we have built an entire suite of tools that will allow us to decipher the role of nucleotide metabolism during normal development and disease, therefore resulting in unprecedented biological insights as well as marked improvements in diagnosis and treatment of patients with cancer.

SHORT TALK #9: **Friday, November 4**

Epithelial-Mesenchymal Plasticity Drives Growth Factor Discordance in Metastatic Breast Cancer

Michael Wendt

Purdue University, West Lafayette, IN

Metastasis is the major cause of breast cancer (BC) lethality and these lesions often display inherent resistance to targeted therapies prescribed based upon molecular characterization of the primary tumor. Induction of epithelial-mesenchymal transition (EMT) is a major contributing factor to BC metastasis and several recent studies have demonstrated that induction and recovery from EMT, or mesenchymal-epithelial transition (MET), selects for a population of cells with stem-like characteristics. We have recently demonstrated that induction of EMT:MET via stimulation and withdrawal of transforming growth factor beta 1 is sufficient to drive the metastasis of EGFR-transformed tumors. Analysis of these metastases that transitioned through an EMT-MET cycle revealed enhanced EGFR-mediated apoptosis, resulting in a progressive loss of EGFR expression and resistance to EGFR-targeted therapy. Concomitant with the loss of EGFR-mediated proliferation, gene expression analyses revealed upregulation of several FGFRs and extracellular matrix proteins. Indeed, we find FGFR1 physically interacts with integrins and FGF signaling is potently increased when metastatic BC cells are cultured within a 3D matrix. To target these metastatic events, we have recently collaborated on the development of FIIN4, a highly potent covalent inhibitor of FGFR kinase activity. Importantly, in vivo application of FIIN4 effectively delays tumor growth in murine and patient derived xenograft models of BC metastasis. Overall, our work demonstrates that an EMT reaction in the primary tumor can lead to discordance in growth factor receptor expression patterns within the metastatic lesion. This molecular “switching” of growth promoting pathways likely contributes to the inherent drug resistance of metastatic tumors.

SESSION 6: Tumor Dormancy **Friday, November 4**

The Role of the Tumor Microenvironment in Tumor Dormancy, Recurrence and Metastasis

Jeffrey Rosen

Baylor College of Medicine, Houston, TX

Despite advances in early detection and targeted adjuvant therapies, breast cancer is still the second most common cause of cancer mortality among women. Tumor recurrence is one of the major contributors to breast cancer mortality. We investigated the mechanisms of tumor dormancy and recurrence in a transplantable Wnt1/inducible fibroblast growth factor receptor (FGFR) 1 preclinical mouse model of breast cancer. Treatment with an FGFR specific inhibitor resulted in rapid tumor regression, leaving a nonpalpable mass of dormant tumor cells organized into a luminal and basal epithelial layer similar to the normal mammary gland, but surrounded by dense stroma with markedly reduced levels of myeloid-derived tumor suppressor cells (MDSCs). Following cessation of treatment the tumors recurred over a period of 1 to 4 months. The recurrent tumors displayed dense stroma with increased collagen, tenascin-C expression, and MDSC infiltration. MDSCs play critical roles in primary and metastatic cancer progression. We discovered that mTOR signaling in cancer cells dictates a mammary tumor's ability to stimulate MDSC accumulation through regulating G-CSF. Inhibiting this pathway or its activators (for example, FGFR) impairs tumor progression, which is partially rescued by restoring MDSCs or G-CSF. Tumor-initiating cells (TICs) exhibit elevated G-CSF. MDSCs reciprocally increase TIC frequency through activating Notch in tumor cells, forming a feed-forward loop. Finally, activation of the epidermal growth factor receptor (EGFR) pathway was observed in recurrent tumors, and inhibition of EGFR with lapatinib in combination with the FGFR inhibitor resulted in a significant delay in tumor recurrence.

SESSION 6: Tumor Dormancy

Breast Cancer Recurrence: When The Dragon Awakes

Lewis Chodosh

Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

For many types of human cancer, residual tumor cells remain following treatment that are not detected by conventional clinical testing. These cells have the ability to survive in a presumed dormant state within tissues for up to 20 years, either as solitary cells or as micrometastases. Ultimately, residual cells re-emerge from this latent state and resume growth, leading to cancer recurrence. Despite the unrivaled clinical importance of these aspects of breast cancer progression, the mechanisms underlying them are largely unknown. Since dormant residual tumor cells constitute the reservoir from which recurrent cancers invariably arise, the lack of therapeutic approaches specifically targeted against these cells – as well as our lack of understanding about their biology – constitute major obstacles to the successful treatment of human cancers. As such, the development of targeted therapies designed to block pathways on which residual tumor cells depend for survival and growth would represent an attractive approach to preventing cancer recurrence.

SESSION 6: Tumor Dormancy

Estrogen Receptor ß5 Inhibits Bcl2L12 Function and Confers Better Breast Cancer Survival

Shuk-Mei Ho[1,2,3,5],

Yuet-Kin Leung[1,2,3], Zoanne Nugent[4], Ming-Tsung Lee[1], Leigh Murphy[4]

[1]Division of Environmental Genetics and Molecular Toxicology, Department of Environmental Health, [2]Center for Environmental Genetics, [3]Cancer Institute, University of Cincinnati College of Medicine, Cincinnati, OH; [4]Department of Biochemistry and Medical Genetics, Manitoba Institute of Cell Biology, Cancer Care Manitoba, University of Manitoba, Manitoba, Canada; [5]Cincinnati Veteran Affairs Medical Center, Cincinnati, OH.

Bcl2L12 was identified as a novel protein partner for estrogen receptor beta5 (ERß5), which counteracts the anti-apoptotic function of Bcl2L12 and promotes chemotherapeutic agent-induced apoptosis in our breast cancer (BCa) study. To elucidate the clinical significance of this finding, we examined the protein level of ERß1, ERß5 and Bcl2L12 in a cohort of 636 BCa patients who were treated with surgery+ radiation and then tamoxifen with tissue microarray-immunohistochemistry (IHC) approach. Relationships of ERß1, ERß5, and Bcl2L12 expression level to BCa-related death and relapse-free survival (RFS) were tested using single (univariate) and multiple (multivariate) predictor statistical models. Along with tumor size and node status, nuclear positivity of Bcl2L12 was significantly associated with shorter survival in patients with BCa-related death and cancer recurrence in all BCa patients or ER negative patients. Interestingly, in ER-positive patients using multivariate analyses, high nuclear ERß5 (nERß5) expression was significantly associated with better survival outcome (BCa-related death or cancer recurrence) whereas cytoplasmic ERß1 (cERß1) was associated with worst survival outcome. Multivariate analyses combined cERß1 and nERß5 predictors suggested that BCa patients with high cERß1 and low nERß5 have two times higher risk with worst survival outcomes (cancer recurrence or combined outcomes) in ER-positive patients. Similarly, low nERß5 together with high nuclear Bcl2L12 expression showed significantly higher risk for BCa-related death or cancer recurrence in a combined multivariate analysis of ER-positive cohort. In conclusion, our study suggested that high nuclear ERß5 and low Bcl2L12 could be the potential prognostic markers for predicting better survival outcome in tamoxifen-treated BCa patients.

This study was supported in part by grants funded by National Institute of Health (R01ES022071, U01ES019480, U01ES020988, and P30ES006096) and the Veteran Affairs (I01BX000675).

SESSION 6: Tumor Dormancy

Models and Mechanisms of Acquired Resistance to Antiestrogene Treatment Strategies Presenter

Jordan, V. Craig

MD Anderson Cancer Center, Houston, TX

Models and Mechanisms of Acquired Resistance to Anti-estrogenic Treatment StrategiesV. Craig JordanBreast Medical OncologyMD Anderson Cancer CenterHouston, Texas 77030Long term adjuvant antihormone therapy using either tamoxifen or an aromatase inhibitor (AI) is the standard of care for post-menopausal patients following a diagnosis of estrogen receptor (ER) positive breast cancer. The duration of endocrine therapy has recently advanced from five years to become 10 years. The strategic use for long term or indefinite antihormone therapy is an example of successful translational research originally conducted in the 1970's. However it would be naive to believe that acquired resistance to antihormone therapy will not occur. Laboratory models have been used to decipher mechanisms and it is now realized that acquired resistance to tamoxifen evolves over time. Laboratory models demonstrate that tamoxifen stimulated growth occurs initially but after five years a vulnerability is exposed which is estrogen induced apoptosis. Cell culture models system of estrogen deprivation mimic acquired resistance to AIs. Intriguingly inhibition of cSrc blocks estrogen induced apoptosis but after two months creates a new cell line referred to MCF7PF. These cells replicate SERM stimulated breast cancer cell growth and grow vigorously with estrogen. Mechanistic studies demonstrate that SERMs stimulate growth occurs non-genomically through IGFR II, but estrogen stimulates tumor cell growth through the classical genomic pathway. Additionally long-term AI therapy can cause mutations in the ER that now result in ER activation and ER closure to form ligand free complex that stimulate tumor cell replication.

SHORT TALK #10: **Friday, November 4**

Impact of Ethnicity-dependent Differences in Normal and Tumor Epigenome on Breast Cancer Progression

Kenneth P. Nephew*,

Manjushree Anjanappa, Poornima Bhat-Nakshatri, Fang Fang, Harikrishna Nakshatri

Indiana University Melvin and Bren Simon Cancer Center; IU School of Medicine, Indianapolis, IN

African American women suffer higher mortality from the aggressive breast cancer subtype, triple negative breast cancer (TNBC), than Caucasian women. Furthermore, basal-like and mesenchymal stem-like TNBCs with elevated intra-tumor heterogeneity are significantly more common in African American women. In contrast, breast cancer in Hispanic women is generally less aggressive. Whether worse outcome in African American women is due to an increased incidence of TNBC or unique biological factors that promote aggressive biology is an important but unresolved challenge in cancer disparities research. To test whether TNBCs in African American women are a mutation-driven, molecularly distinct disease or have inherently aggressive biology driven by disparities, we carried out an extensive phenotypic, epigenomic, and transcriptomic characterization of normal breast epithelial cells samples of African American, Caucasian, and Hispanic women. By using a unique culturing system for propagating normal epithelial cells of different lineages, including differentiated-luminal, luminal-progenitor, and stem cells from the breast core biopsies of healthy African American, Caucasian, and Hispanic women, we demonstrated selective enrichment of multi-potent progenitor cells in normal African American breast. We showed a gene expression pattern overlapping mesenchymal-like subtype TNBC, indicating that African American TNBCs originated from a distinct population of breast epithelial cells with inherent mesenchyme-like (cancer-prone) features. Epigenetic profiling of cancer-prone luminal progenitor cells and less-cancer prone differentiated cells revealed striking ethnicity-dependent differences in DNA methylation and gene expression affecting cellular differentiation and response to stemness and de/differentiation-associated microenvironmental cues. Cells typically considered to be differentiated, based on the cell surface marker profile CD49f-/EpCAM+, showed remarkable ethnic differences in DNA methylome and transcriptome. For example, presumably differentiated cells in African American women retained expression of several stem/progenitor cell-enriched genes such as ZEB2 but had lower GATA3 expression, a key component of the master cell-type specific transcription factor network linked to luminal progenitor differentiation and response to steroid hormone estradiol. In addition, pathways associated with tight junction signaling and HIPPO signaling, both required for pregnancy-associated differentiation, were hypermethylated only in CD49f- /EpCAM+ cells of African American women. Cells isolated from healthy Hispanic women breast lacked those features and moreover displayed differentiated (less-cancer prone) characteristics, such as extensive methylation of pluripotency-associated genes and Wnt/ß-catenin signaling in luminal progenitor cells. In summary, our integrated DNA methylome and transcriptome studies of purified subpopulation of cells showed that phenotypically differentiated cells of African American women express genes associated stemness, cancer-prone luminal progenitors, and plasticity compared to similar cells from Caucasian and Hispanic women. We suggest that differences in normal breast epithelial cell hierarchy of ethnic groups may impact tumor evolution/ heterogeneity, and ethnic differences exist in differentiation of stem to mature cells. In addition, identifying ethnicity-dependent markers of breast cancer risk may call for reassessment of clinically used prognostic/predictive markers that consider ethnicity as a confounding factor.

SHORT TALK #11: **Friday, November 4**

A Rosetta Stone for Deciphering Breast Cancer Genomics to Identify Molecular Targets & Diagnostics

James L. Wittliff

Biochemistry & Molecular Genetics, Institute for Molecular Diversity & Drug Design, University of Louisville, Louisville, KY

Forecasting clinical behavior and therapeutic response of human cancer currently utilizes a limited number of tumor markers in combination with characteristics of the patient and their disease. Medically relevant genomic and proteomic test development utilizes a troika consisting of human tissue specimens collected and stored under specialized conditions with annotated clinical records, isolation of distinct cell types with technologies such as laser capture microdissection and analyses of macromolecules with protease-free and RNase-free protocols to generate gene and protein expression profiles. Although few tumor markers and new molecular targets exist for evaluation of a cancer patient's prognosis and therapy selection, the wealth of information derived from the Human Genome Project provides greater opportunities to develop more precise tests for diagnostics, prognostics, therapy selection and monitoring. Clinically relevant genomic and proteomic test development using de-identified human tissue specimens from our extensive IRB-approved BioRepository requires specialized collection, handling and cryopreservation methods for generating reliable analyses. Although global gene expression assays of intact cancer biopsies are utilized to distinguish patterns, validation of mRNA expression of specific gene sets by techniques such as quantitative PCR is essential using well characterized samples. Non-destructive procurement of pure cell populations from frozen and formalin-fixed, paraffin-embedded tissues by Laser Capture Microdissection and optimized methods for RNA and protein analyses enhance identification of candidate molecular targets for development of drugs and diagnostics. These approaches and Next Generation Sequencing technologies must be complemented by well annotated records of patient characteristics, tissue pathology and clinical outcome. Collectively, genomic and proteomic strategies for accessing human cancers using intact tissue sections and LCM-procured cells are providing insights into new molecular diagnostics and targets for drug design as well as an improved understanding of the molecular basis of clinical behavior and therapeutic response.

POSTERS

1

Macrophage-specific Ron Signaling Supports Breast Cancer Stem Cell Maintenance

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Current treatment against breast cancer (BC) targets rapidly-proliferating cells. However, tumor recurrence and therapeutic resistance persist, highlighting their lack of effectiveness. A small population within breast tumors, named Breast Cancer Stem Cells (BCSCs), has been shown to be essential for tumor development, recurrence, and metastasis due to their increased self-renewal, survival, migration, and therapeutic resistance. BCSC properties are stimulated by intrinsic regulatory mechanisms and extrinsic mechanisms induced by cells in the tumor microenvironment (TME), such as macrophages. The Ron receptor tyrosine kinase is primarily expressed in macrophages and epithelial cells and is found overexpressed in human BCs, being associated with increased metastasis and poor prognosis. Recently, we demonstrated that Ron drives BCSC maintenance by intrinsically regulating their self-renewal and tumorigenic potential as well as by regulating the TME. However, it is unknown whether Ron signaling in macrophages affects BCSC phenotypes and tumor growth. Tumor kinetics, flow cytometry analyses for BCSC markers, and mammosphere formation assays for tumors from mice with or without a conditional deletion of Ron in macrophages show that Ron loss in macrophages diminishes tumor growth, BCSC numbers, and their self-renewal ability. Further mammosphere formation analyses also demonstrate that macrophage-specific Ron signaling enhances BCSC self-renewal through the production of a soluble factor. Overall, our data provides the first evidence showing that Ron expression in macrophages stimulates BC growth and BCSC maintenance through the production of a secreted factor. These studies suggest Ron as potential therapeutic target to effectively eradicate BCSCs and improve the outcome of BC patients.

2

Expression of the DEK Oncogene Promotes M2 Polarization and Iron Recycling in Tumor Associated Macrophages

Nicholas A. Pease, Jonathan Cheek, and Lisa M. Privette Vinnedge

Division of Oncology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

The chromatin-remodeling DEK oncogene is highly expressed in >60% of breast cancers, particularly in triple-negative breast cancers, and is associated with poor clinical outcome. Previously, using human breast cancer (BC) cell lines and the MMTV-Ron mouse BC model, we demonstrated that DEK promotes proliferation and metastasis and supports the BC stem cell population due, in part, to upregulated Wnt expression and b-catenin activation. To investigate additional drivers of DEK-induced tumorigenesis, we performed RNA-Seq on DEK proficient and deficient cells, which identified immune signaling processes. We thus investigated the immune cell infiltration of tumors from MMTV-Ron/Dek+/+ and MMTV-Ron/Dek-/- mice. Dek+/+ tumors demonstrated decreased tumor associated macrophage (TAM) infiltration that had an iron-recycling phenotype (M2-like polarization), whereas TAMs from Dek-/- tumors retained iron (M1-like polarization). M1 macrophages are typically associated with inflammation whereas M2 macrophages support tissue remodeling, angiogenesis, and tumor promotion. Dek+/+ and Dek-/- bone marrow-derived macrophages (BMDM) had no inherent difference in polarization tendency. However, BMDM exposed to conditioned media from Dek-expressing cancer cells recapitulated the iron recycling phenotype observed in vivo, and had increased expression of M2 markers ferroportin, CXCR4, and VEGF, and an elevated ARG1hi/NOSlo population. Small molecule inhibitors implicated secreted Wnt ligands from the cancer cells, and downstream C/EBP transcriptional activity in the macrophages, as possible mechanisms. This suggests that the poor clinical outcome of high DEK expressing breast cancers may be due to not only cell intrinsic factors, but also cell extrinsic factors, such as the tumor-promoting M2 polarization of tumor associated macrophages.

3

FIP200 Controls Neural Stem Cell Differentiation by Regulating Microglia Infiltration Through a p53-independent and Non-Cell Autonomous Mechanism

Wang, Chenran; Yeo, Syn Kok; Guan, Jun-lin

Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH

Recent studies have shown important roles for autophagy genes in the regulation of different tissue stem cells including neural stem/progenitor cells (NSCs). However, little is known about whether autophagy can regulate NSCs through cell-extrinsic mechanisms or cross-talk between NSCs and microglia in neurogenic niches. Here, we show that deletion of an essential autophagy gene FIP200 in NSCs increased expression of Ccl5 and Cxcl10 in a p53-independent manner in NSCs, which mediated increased migration and infiltration of microglia into the subventricular zone of both FIP200hGFAP cKO and FIP200;p53hGFAP 2cKO mice. The more abundant microglia exhibited an activated M1 phenotype, consistent with their potential to inhibit differentiation of FIP200-null NSCs. Blocking either the elevated infiltration of microglia or their activation significantly rescued the deficient differentiation of FIP200-null NSCs from FIP200;p53hGFAP 2cKO mice. Lastly, we showed that increased chemokine expression in FIP200-null NSCs was induced by abnormal p62 aggregate formation and its activation of NF-kappa B signaling. Our results suggest that autophagy plays a crucial role in regulating neurogenesis and restricting local immune response in postnatal NSCs through a non-cell-autonomous mechanism.

4

Suppression of Anti-Tumor immunity by E2F3

Safiya Khurshid, Hui Wang, Sooin Bae, Gustavo Leone

Department of Cancer Biology and Genetics, College of Medicine, Department of Molecular Genetics, College of Biological Sciences, and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio

Activation of the HER2-CCND1-CDK4 pathway in breast cancer is believed to culminate in the loss of RB function, release of E2F transcriptional activity and uncontrolled tumor cell proliferation. Using conditional gene knockout strategies in mice we demonstrate that ablation of the E2f3 family member delays HER2 mammary tumorigenesis and promotes regression of established HER2-tumors. Surprisingly, the loss of E2f3 affected neither proliferation nor apoptosis of tumor cells, but rather resulted in activation of p19ARF, p53 and p53 targets such as Lif and the execution of anti-tumor immune responses. We can abrogate this delay in tumorigenesis caused by loss of E2f3 when we knockout the CD8 positive cells of the immune system. These findings shift the current paradigm of the RB-E2F axis in breast cancer beyond cell cycle control, to include oncogene-specific immune-suppressive functions.

5

The Effects of Cholesterol and Its Metabolites on the Metastasis of Breast Cancer to Bone

Sayyed Hamed Shahoei[1], Heidi Phillips[2], Erik R. Nelson[1,3]

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Bone is the most common distal metastatic site of breast cancer (BC) metastasis. Successful colonization requires a decrease in local bone quality, either by a cancer cell-driven osteolytic cycle or by host deficiency (i.e. osteoporosis). Interestingly, relapse free survival time is significantly shortened in hypercholesterolemic BC patients, while cholesterol synthesis inhibitors (statins) are protective, and statin therapy protects from age-related bone loss. Thus, we hypothesized that hypercholesterolemia facilitates bone-specific BC metastasis by decreasing bone quality, creating a favorable microenvironment. Supporting this hypothesis are our results indicating that a high-cholesterol diet increases the colonization of bone-tropic, estrogen receptor (ER) negative MDA-MB-231 cells. 27-hydroxycholesterol (27HC) has previously been implicated in mediating the negative effects of cholesterol on bone by behaving as both a selective estrogen receptor modulator (SERM) and a partial agonist of the liver X receptor (LXR). Therefore, we are currently conducting follow-up experiments to determine the (1) extent to which the high-cholesterol-diet increase in bone-specific metastasis is due to 27HC and (2) whether this effect is mediated by ERs and/or LXRs. Furthermore, since the majority of breast cancer patients present with ER+ disease, we have developed a model of ER+ BC metastasis to the bone in immunocompetent mice. We will confirm the effects of cholesterol/27HC in this model. Our results support the idea that elevated cholesterol increases BC metastasis to the bone, likely via 27HC, providing rationale for the development of statins, inhibitors of 27HC synthesis, and ER or LXR modulators for the prevention of bone metastasis.

6

Patient HER3 Mutations Render Human Mammary Epithelial Cells Resistant to HER2 Inhibition

Garrett, Joan

University of Cincinnati

We are examining the role of naturally occurring HER3 mutations in the context of HER2-driven and ER-driven breast cancers. We introduced a series of HER3 mutations (F94L, G284R, D297Y, D313H, K329T, T355I, L792V, E1261A), identified in breast cancer patients, using site-directed mutagenesis. Stable cell lines were generated in MCF10A/HER2 and ER+ MCF7 and T47D breast cancer cells using lentiviral transduction. We identified several HER3 mutations that had higher cell proliferation than wild-type HER3 in MCF10A/HER2 cell including G284R, D297Y, T355I, and E1261A. These mutations notably had increased phosphorylated HER3 compared to cells expressing wild-type HER3. Furthermore, these mutations counteracted the effect of the HER2 inhibitor lapatinib. In the context of ER+ breast cancer we observed the T355I and other mutations to have statistically increased proliferation compared to wild-type HER3. Experiments are ongoing to determine if these mutations render ER+ tumor cells resistant to antiestrogen therapy. In parallel experiments, we tested the effect of knocking down HER3 in tumor cell lines that harbor endogenous HER3 mutations. The HER3 K742E mutation occurs endogenously in the IGROV-1 (ovarian) and P262H & V104M in SNU-407 (colorectal), A232V in SNU-1040 (colorectal), N126K and R667H in HCT-15 (colorectal) cancer cell lines. We transfected the cells with either HER3 or control siRNA and determined that each cell line had a reduction in proliferation with knockdown of HER3. These data suggest the potential for HER3 mutations to be oncogenic.

7

Proteomic Profiling Identifies Key Coactivators Utilized by Mutant ER-alpha Proteins to Promote Ligand-Independent Transcription and Cell Growth

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Approximately 70% of breast cancers are estrogen receptor alpha (ER-alpha)-positive and are driven by estrogen. Targeting ER-alpha with endocrine therapies provides effective adjuvant treatment with reductions in patient recurrence approaching 50%. Still, many tumors acquire resistance, and in ~20% of these cases, gain-of-function mutations in the ER-alpha gene (ESR1) are implicated. Amino acid substitutions in the receptor's ligand binding domain (LBD) result in receptors displaying ligand-independent activity and endocrine therapy resistance. In addition, rarer translocations of ESR1 to other genes create fusion proteins that lack the LBD and cannot be targeted with anti-estrogens. Our studies focus on the most prominent of the ER-alpha LBD mutants, Y537S and D538G, and the ESR1-YAP1 fusion protein. We propose that therapeutic strategies designed to target coactivators (CoAs) binding a mutant receptor offer a unique approach to enhance treatment of recurrent disease. To define the set of CoAs binding a particular mutant receptor, a mass spectrometric (MS) approach to profile the CoA "complexome" for each mutant ER-alpha protein was performed. MS data of CoA recruitment to each mutant was compared to the wild-type (WT) receptor to identify potential new targets. We observed specific CoAs displaying enhanced binding to each mutant ER-alpha receptor. Importantly, inhibiting these CoA candidates (with small molecule inhibitors and/or siRNA) reduced the ability of the different ERa mutants to activate ERE-driven gene transcription and to promote breast cancer cell growth. Thus, inhibition of distinct CoAs may be a promising new therapeutic strategy to inhibit growth of ER-alpha-mutant expressing breast cancers.

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An Orphan Nuclear Receptor NR2E3 is Associated with Good Prognosis of Liver Cancer by Impending LSD1-dependent Repression of AHR Expression

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The aryl hydrocarbon receptor (AHR) plays crucial roles in inflammation, metabolic disorder, and cancer. However, the molecular mechanisms regulating AHR expression remain unknown. Here, we found that NR2E3 maintains AHR expression, and forms an active transcriptional complex with transcription factor Sp1 and coactivator GRIP1. NR2E3 loss promotes the recruitment of LSD1, a histone demethylase of histone 3 lysine 4 di-methylation (H3K4me2), to the AHR gene promoter region, resulting in repression of AHR expression. AHR expression and responsiveness along with H3K4me2 were significantly reduced in the livers of Nr2e3rd7 (Rd7) mice that express low NR2E3 relative to the livers of wild-type mice. SP2509, an LSD1 inhibitor, fully restored AHR expression and H3K4me2 levels in Rd7 mice. Lastly, we demonstrated that both AHR and NR2E3 are significantly associated with good clinical outcomes in liver cancer. Together, our results reveal a novel link between NR2E3, AHR, and liver cancer via LSD1-mediated H3K4me2 histone modification in liver cancer development.

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GTP Metabolic Switch Drives Cellular Mass Anabolism for Glioblastomagenesis

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Rapidly growing cells, like tumor cells, need a vast amount of energy to match their high metabolic demand. Guanine triphosphate (GTP), a purine nucleotide, is one of the major cellular metabolites and serves as a building block for RNA and DNA, as well as an energy source to drive cellular activities such as intracellular trafficking, cell migration, and translation. However, how cancer cells regulate GTP energy levels to adapt for their high demands remains largely unknown. Using biochemical and immunohistological analyses in mouse glioma models and human glioblastoma specimens, we have discovered that the expression levels of a key enzyme in GTP metabolism are dramatically changed during glioblastoma formation. Our CE-MS/MS (Capillary Electrophoresis and tandem mass spectrometry)-based flux analysis has unveiled a dichotomous activation of a specific GTP-biosynthetic pathway in glioblastoma, compared to normal glia—the origin of glioblastoma. Genetic, as well as pharmacological, inhibition of glioblastoma-specific GTP metabolism abolishes glioblastoma growth and its tumorigenic activity. Mechanistically, we have found that the GTP metabolic switch in glioblastoma is required for mass anabolic reactions. Collectively, our data reveal that glioblastoma activates the GTP switch for metabolic adaptation and GBM progression, which is likely to fulfill the over 100 years of missing knowledge between GTP biosynthesis and cell growth.

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Endothelial Cell-specific Foxf1 has a Tumor-suppressive Role in Lung Cancer

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Lung cancer is the most common cancer in the world with 1.8 million patients diagnosed every year. Current treatments include surgical resection of early-stage lung cancer, and drug therapies that target driver mutations for advanced stages of the disease. Despite advances in the field of therapeutics, the 5-year survival rate of patients with advanced stage disease remains less than 20%, making lung cancer the most common cause of cancer-related mortality. The lung tumor microenvironment has been shown to play an important role in tumor progression, making it an attractive target for anti-cancer therapy. Endothelial cells, a crucial component of the tumor microenvironment, are reprogrammed to support tumor growth. However, the transcriptional programs that regulate their pro-tumorigenic properties remain understudied. Forkhead box F1 (Foxf1) is a transcription factor expressed in lung endothelial cells and has been shown to play an important role in lung development. We recently identified that Foxf1 expression is lost from the endothelial cells within tumors of patients with invasive lung adenocarcinoma. Deletion of one allele of Foxf1 from endothelial cells promoted lung tumor growth in an orthotopic lung cancer model in mice. This suggests that endothelial foxf1 plays a tumor-suppressive role. This study aims at understanding the mechanism by which endothelial foxf1 plays a protective role in lung tumor progression.

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Metabolic Alterations to the Pentose Phosphate Pathway (PPP) Result in Survival Advantages for VHL(-) Clear Cell Renal Cell Carcinoma (ccRCC)

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The PPP plays important cellular roles in the production of ribonucleic acids, regulation of oxidative stress and mainting carbon homeostasis. Further its activity correlates with poor prognosis in patients with ccRCC. However, there is conflicting literatrue regarding the roles of the two PPP branches, oxidative vs. non-oxidative in the generation of PRPP which is essential for de novo nucleotide sythesis pathway. To determine the contributions of each branch to PPP outputs we generated 786-O VHL (-) RCC cells with knockdowns (KD) of key enzymes Glucose-6-phosphate dehydrogenase (G6PDH) from the oxidative branch, or Tranketolase (TKT) from the non-oxidative branch. Analysis of steady-state metabolites was performed using liquid chromatography coupled with mass spectrometry. G6PDH-KD resulted in a significant decrease in 6-phosphogluconate, an intermediate of the the oxidative branch and PRPP as well as, decreased ratios of GSH:GSSG. In contrast, TKT-KD showed an accumulation of metabolites produced in the non-oxidative branch with an increase in PRPP. No significant differences were observed in glucose consumption or glycolysis rates in either cell line when compared to the control. The data suggests that the oxidative branch has an essential activity in the generation of nucleic acids, maintenance of redox potential and that the non-oxidative branch may limit this activity. Surprisingly, the G6PDH-KD cells show a long-term survival benefit when grown under glucose deprivation and potentially indicates a survival mechanism mediated by the nucleotide salvage pathway.

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FGFR Signaling Maintains a Drug Persistent Cell Population Following Epithelial-Mesenchymal Transition

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Epithelial-mesenchymal transition (EMT) expedites the development of resistance to targeted therapies in cancer. However, the mechanisms of EMT-mediated drug resistance are not well understood. Therefore, we conducted long-term treatments of human epidermal growth factor receptor-2 (Her2)-transformed breast cancer cells with either the EGFR/Her2 kinase inhibitor, Lapatinib or TGF-ß, a known physiological inducer of EMT. Both of these treatment regimes resulted in robust EMT phenotypes, but upon withdrawal a subpopulation of TGF-ß induced cells readily underwent mesenchymal-epithelial transition, whereas Lapatinib-induced cells failed to reestablish an epithelial population. The mesenchymal population that remained following TGF-ß stimulation and withdrawal was quickly selected for during subsequent Lapatinib treatment, manifesting in inherent drug resistance. The Nanostring cancer progression gene panel revealed a dramatic upregulation of fibroblast growth factor receptor 1 (FGFR1) and its cognate ligand FGF2 in both acquired and inherent resistance. Mechanistically, FGF:Erk1/2 signaling functions to stabilize the EMT transcription factor Twist and thus maintain the mesenchymal and drug resistant phenotype. Finally, Lapatinib resistant cells could be readily eliminated using recently characterized covalent inhibitors of FGFR. Overall our data demonstrate that next-generation targeting of FGFR can be used in combination with Her2-targeted therapies to overcome resistance in this breast cancer subtype.

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C-terminal Domain of Complexin-1 Localizes to Highly Curved Membranes

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In pre-synaptic nerve terminals, complexin regulates spontaneous “mini” neurotransmitter release and activates Ca2+-triggered synchronized neurotransmitter release. We studied the role of the C-terminal domain of mammalian complexin in these processes using single-particle optical imaging and electrophysiology. The C-terminal domain is important for regulating spontaneous release, but it is not essential for evoked release in neuronal cultures and in vitro. It interacts with membranes in a curvature dependent fashion similar to a previous study with worm complexin. The curvature sensing value of the C-terminal domain is comparable to that of a-synuclein. Upon replacement of the C-terminal domain with membrane-localizing elements, localization to the synaptic vesicle membrane, but not to the plasma membrane, results in suppression of spontaneous release in neurons. Membrane localization had no measurable effect on evoked postsynaptic currents of AMPA-type glutamate receptors, but mislocalization to the plasma membrane increases the synchronous decay time constant of NMDA-type glutamate receptor evoked postsynaptic currents.

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Ron Receptor Signaling Promotes Resistance to Androgen Ablation Therapy in Prostate Cancer

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Prostate cancer (PCa) is the second leading cause of cancer related deaths in men. Many deaths result from resistance to androgen ablation therapy, defined as Castration Resistant PCa (CRPC), where median survival is only 19 months. CRPC often develops by reactivation of the Androgen Receptor (AR). We have strong data suggesting that the Ron tyrosine kinase activates AR in PCa to promote resistance to androgen ablation therapy. Prior studies established that Ron is highly expressed and plays a functional role in promoting PCa. To address Ron's role in CRPC, human tissues were analyzed and Ron was observed to be highly expressed in all CRPC samples. Overexpression of Ron in androgen sensitive PCa cell lines used in murine models of CRPC show that Ron overexpression is sufficient to drive CRPC in vivo. Ron overexpressing prostate tumors have elevated AR activation and require AR for growth under androgen deprivation. Enhanced macrophage recruitment was observed in Ron overexpressing tumors and castration resistant growth of Ron overexpressing cells was inhibited in vivo when macrophages were depleted in combination with castration therapy, suggesting a non-cell autonomous role for macrophages in promoting Ron mediated CRPC. Further studies focused on the role of Ron in CRPC may provide the scientific underpinnings for targeting Ron signaling to combat resistance to androgen ablation therapy.

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FAK is Required for the Survival of Tumor Cells in MMTV-Wnt1 Driven Basal-like Mammary Tumors

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Breast cancer is a heterogeneous disease. Stratification of patients based on the subtype of breast cancer is a key to successful treatment of breast cancer. Focal Adhesion Kinase (FAK), a cytoplasmic tyrosine kinase is over expressed and activated in several cancers including breast cancer. Our earlier studies have shown inhibition of FAK in MMTV-PyMT mouse mammary tumors that are classified as Luminal B subtype, delays tumor onset and reduces tumor growth. To address whether inhibition of FAK would be beneficial in basal-like mammary tumors, we generated a conditional deletion of FAK and a knock in mutation of FAK lacking its kinase activity, in MMTV-WNT1 mouse model, which classifies as basal-like. Similar to PyMT mammary tumors we found that loss of FAK or its kinase function delays tumor onset and tumor growth of basal like WNT1 mammary tumors. However unlike the PyMT tumors, the reduced tumor growth in WNT1 model is not due to decreased proliferation. Interestingly loss of FAK activity in WNT1 tumors results in accumulation of cleaved caspase 3, suggesting that loss of FAK activity results in compromised tumor cell survival. When we investigated the pathways through which FAK could affect survival, we found that loss of FAK activity reduces activation of AKT. Reduced AKT activation induces the expression of pro-apoptotic genes. In summary our studies show that in a basal-like tumor model, FAK is required for survival of the tumor cells. Hence inhibition of FAK could be beneficial for elimination of basal-like tumor cells.

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Nuclear FAK Controls VEGFR2 Transcription in Angiogenesis

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Angiogenesis is a complex biological process which plays an essential role in embryogenesis, the homeostasis of adult animals, and various diseases including coronary heart disease, age-related macular degeneration, diabetes and cancer. Endothelial cells (ECs) are central players in angiogenesis, and their responses to extracellular stimuli such as VEGF are crucial in angiogenesis during embryogenesis and in adult organisms. Of several VEGF receptors, VEGFR2 has been identified as a principal mediator of various physiological and pathological effects of VEGF on ECs, including proliferation, migration, survival and permeability. FAK is a major mediator of signal transduction by integrins and also participates in signaling by VEGFR2 in ECs. Previous in vitro studies have shown that FAK is critical for EC migration, proliferation and survival. Recent studies using EC-specific FAK conditional KO and kinase-defective (KD) mutant knockin mouse models demonstrated both the kinase-dependent and kinase-independent function of FAK in embryonic angiogenesis. However, the potential role of FAK in adult angiogenesis is still controversial. Moreover, the underlying mechanisms, especially the downstream signaling pathways of FAK in the regulation of EC function and angiogenesis in adult organisms, remain to be characterized. Here we found FAK directly controls VEGFR2 expression in ECs. Mechanistically, in ECs, nuclear FAK is associated with chromatin as an integral component of VEGFR2 transcription factor complex, and controls VEGDR2 mRNA transcription. Its kinase activity is also essential for this process. Therefore, our results provide significant insights into the signaling mechanisms FAK in angiogenesis that may contribute to future design of more effective angiogenesis related therapy.

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Study on the Role of Fip200 in Lymphangiosarcoma Development and Progression

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Lymphangiosarcoma is a rare malignant vascular tumor which is formed from aberrant proliferation of endothelial cells (ECs). Our previous study revealed that both sustained mTORC1 signaling and increased VEGF autocrine are required for lymphangiosarcoma development. However, the role of autophagy in lymphangiosarcoma development and progression is unclear. Autophagy gene Fip200 (FAK family interacting protein of 200 kDa) was reported to be associated with some cancer development. Our previous study has established a lymphangiosarcoma mouse model. Here we use a transformed murine Tsc1 ECs, combined with MS1 cell line, to study the role of Fip200 in lymphangiosarcoma. We found that Fip200 knockdown of Tsc1 ECs impaired tube formation of the tumor cells, but did not affect tumor cells migration and proliferation. However, Fip200 and Tsc1 double knockdown of MS1 cells blocked cell cycle progression compared with Tsc1 single knockdown. Further study revealed that Fip200 knockdown suppressed mTORC1 signaling activation, indicated by downregulating p70 S6K and 4E-BP1 phosphorylation, as well as inhibited the expression of HIF1a and VEGF under glucose starvation. Moreover, Fip200 knockdown showed substantial resistance to glucose starvation-induced apoptosis and increased cell survival. In addition, our data showed that Fip200 knockdown of Tsc1 ECs inhibited AMPK activation by downregulating AMPK and its downstream effector ULK1 phosphorylation at serine 555. Glucose uptake was also found to be suppressed following Fip200 knockdown of MS1 cells. Collectively, our data suggested that Fip200 could contribute to lymphangiosarcoma development and progression via multiple mechanisms.

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MED1 LxxLL Motifs Play Critical Roles in HER2-driven Breast Tumorigenesis through the IGF-1 Pathway

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MED1 is a key ER transcriptional coactivator that directly interacts with ER through its LxxLL motifs to regulate target gene expression. We have generated a mutant knockin (MED1KI/KI) mouse model with both MED1 LxxLL motifs mutated. Unexpectedly, we found that MED1 mutations did not affect the overall fertility and survival of the mice, but played a rather tissue-, cell-, and gene-specific role in the pubertal mammary gland development. Interestingly, the MED1 gene is located in the HER2 amplicon and co-amplifies with HER2 in human breast cancer. We and others have further established MED1 as the key crosstalk point for HER2 and ER signaling pathways in breast cancer. However, whether MED1 LxxLL motifs play a role in HER2+ breast cancer tumorigenesis still remains unknown. In this study, we have crossed our MED1KI/KI mice with MMTV-HER2 mammary tumor model. Significantly, we observed greatly delayed tumor onset, impaired tumor growth and reduced lung metastasis in MMTV-HER2/MED1KI/KI mice. Consistent with those, there is a marked decrease in cell proliferation, widely spread tumor necrosis and reduced blood vessel formation in these tumors. Moreover, we found tumor stem cell (CSC) formation was significantly impaired by MED1 mutation. Further mechanistic studies revealed that MED1 mutations function through directly deactivating the ER downstream IGF-1 signaling pathway. Importantly, these findings were further confirmed using both human breast cancer cell lines and clinical samples. Overall, our study supports a critical role for MED1 LxxLL motifs in HER2-driven breast cancer tumorigenesis and its potential use as a tissue-specific therapeutic target.

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Overcoming Tamoxifen Resistance of Human Breast Cancer by Targeted Gene Silencing

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Most breast cancers (~75%) express estrogen receptor (ER), and anti-estrogen tamoxifen has been widely used as the first line adjuvant therapy in their treatment. Unfortunately, up to half of all ER-positive tumors have intrinsic or acquired endocrine therapy resistance. Our recent studies have revealed that ER coactivator MED1 plays a critical role in tamoxifen resistance through crosstalk with HER2. Herein, we assembled a novel three-way junction pRNA-HER2apt-siMED1 nanoparticle to target HER2-overexpressing human breast cancer via a HER2 RNA aptamer and silence MED1 expression. These nanoparticles are ultra-compact and very stable under RNase A, 8 M urea, serum and PBS conditions. Importantly, pRNA-HER2apt-siMED1 nanoparticles could specifically bind to HER2-positive breast cancer cells, efficiently deplete MED1 expression and decrease ER-mediated gene transcription, whereas mutation of the HER2 RNA aptamer within these nanoparticles abolished their functions. Moreover, pRNA-HER2apt-siMED1 nanoparticles could not only reduce the growth, metastasis ability and mammosphere formation of HER2-positive breast cancer cells, but also effectively sensitize them to tamoxifen treatment. These nanoparticles also exhibited excellent biosafety with strong ability to accumulate and penetrate into HER2-overexpressing tumors after systemic administration to orthotopic xenograft mouse models. Most importantly, our findings demonstrated that in addition to their ability to greatly inhibit tumor growth and metastasis, pRNA-HER2apt-siMED1 nanoparticle treatment with tamoxifen dramatically reduced the breast cancer stem cell population. In all, we have been able to generate highly promising multifunctional pRNA nanoparticles capable of specifically targeting HER2-overexpressing human breast cancer to silence MED1 and overcome tamoxifen resistance.

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Targeting Breast Cancer Metastasis By Novel RNA Aptamers Against MED1 LxxLL Motifs

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Metastatic breast cancer (MBC) is the leading cause of cancer-related death in women worldwide. Approximately 75% of breast cancer cases are estrogen receptor positive (ER+) and both experimental and clinical evidence support a key role for ER signaling in metastasis. MED1 is the ER-interacting subunit of the Mediator transcriptional coactivator complex bridging ER to RNA Polymerase II. MED1 interacts with ER directly through its two classical LxxLL motifs to mediate target gene transcription. Our recent research has demonstrated that when these motifs are mutated in mice, they are grossly normal other than defects in pubertal mammary gland development. We have further crossed these MED1 LXXLL-mutant mice with mammary tumor-prone MMTV-PyVT mice and observed significant loss in tumor growth and metastasis. In this study, we have isolated novel RNA aptamers to target these MED1 LxxLL motifs and disrupt its function. RNA aptamers are an emerging class of diagnostics and therapeutic agents with the ability to bind specifically to a desired target, rivaling antibodies and small molecules. In particular, they are highly stable in vivo, capable of carrying a number of functional moieties, and do not elicit unwanted immune response. Currently, we have 8 aptamers candidates that can specifically bind to the MED1 LxxLL motifs and disrupt the ER-MED1 interaction. Importantly, we have found at least one such aptamer (aptamer “SP”) that cannot only significantly inhibit ER-target gene expression, tumor growth, migration and invasion in vitro, but also block tumor growth in an orthotopic xenograft mouse model.

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Exploiting the EGFR Paradox for the Treatment of Metastatic Breast Cancer

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Metastasis is responsible for the vast majority of patient mortality in breast cancer (BC). Amplification of epidermal growth factor receptor (EGFR) is capable of transforming mammary epithelial cells, and contributes to several early events in tumor invasion and dissemination. However, clinical trials evaluating several different anti-EGFR therapies have not been successful. To further investigate this we have developed a progression series of cell-lines that demonstrate in the later stages of metastasis the function of EGFR paradoxically shifts from a proliferative factor to an inhibitor of cell growth. Herein, we show that EGF stimulation of apoptosis in metastatic BC cells is concomitant with a corresponding increase in EGF-induced activation of STAT1. Moreover, blockade of Mek:Erk1/2 signaling downstream of EGFR (using Trametinib) enhances EGF-induced STAT1 phosphorylation and greatly augments EGF-induced apoptosis in metastatic BC cells. Importantly, the EGF+Trametinib combination also triggered apoptosis and STAT1 activation in nonmetastatic BC cells that normally respond to EGF in a proliferative fashion. These findings are not restricted to our model system as human-derived BC cell-lines MDA-MB-468 and BT-20 similarly activated STAT1 and underwent apoptosis upon dual treatment with EGF and Trametinib. Importantly, this treatment combination did not induce apoptosis in normal mammary epithelial cells. Given this in vitro data, we are currently testing the efficacy of EGF agonism in combination with Trametinib in vivo using metastatic BC cell-lines. These exciting findings point to EGFR agonism with Trametinib as a possible in vivo therapeutic strategy to eradicate both primary and metastatic BC cells by activation of STAT1.

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WNT7b Promotes Cell Proliferation in a DICER1-deficient Murine model of the Childhood Lung Tumor, Pleuropulmonary Blastoma

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Pleuropulmonary blastoma is the most common pediatric lung tumor, which is comprised of epithelial-lined cysts with surrounding expanded mesenchyme that can progress to lethal sarcomas. Germline loss-of-function heterozygous mutations in DICER1, a critical component in the maturation of microRNAs, were discovered in 66% of PPBs. Interestingly, DICER1 protein is absent in PPB epithelium, but still present in the mesenchyme, suggesting mesenchymal expansion occurs via paracrine signaling. Our previously published work demonstrates that DICER1 loss in the developing lung epithelium is sufficient for PPB initiation, including mesenchymal expansion, but the molecular mechanisms underlying the phenotypes remain undefined. Utilizing a candidate gene approach we identified WNT7b, whose expression is restricted to the lung epithelium and is a key regulator of epithelial to mesenchymal signaling, was increased with DICER1-ablation despite unaltered expression of other well-known lung signaling cascades. We hypothesize that DICER1 loss stimulates WNT7b dependent signaling to promote epithelial and mesenchymal phenotypes in PPB pathogenesis. In support of this hypothesis, increased Wnt7b expression in DICER1 deficient lungs is associated with upregulation of downstream signaling molecules, such as N-Myc, in a temporally dependent manner. This is consistent with the timing of DICER1 ablation and appearance of DICER1-dependent phenotypes. A mouse model wherein Dicer1 and Wnt7b are ablated in the lung epithelium was established to assess if Wnt7b loss is sufficient to rescue PPB phenotypes in vivo. Reducing Wnt7b levels in DICER1-deficient lungs restored mesenchymal proliferation to wild-type levels providing evidence that WNT7b is a mediator of DICER1-dependent proliferation in PPB.

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Ron driven PCNA Phosphorylation In Vivo Mediates Mammary Tumorigenesis and Metastasis

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Consistently, breast cancer has remained one of the most common cancers diagnosed in women despite major advances in screening; understanding novel molecular tumorigenic processes in clinically relevant models is therefore imperative. Proliferating Cell Nuclear Antigen (PCNA) is a trimeric nuclear protein essential in many cellular processes. Forming the sliding clamp during DNA replication, activation of PCNA through phosphorylation is a crucial molecular process for cellular proliferation and within the context of breast cancer, phospho-Tyr211 has been shown to induce proliferation. The Ron receptor tyrosine kinase also plays important roles in proliferative processes such as wound healing, but is upregulated in many cancers including breast cancer. Ron overexpression in human breast cancers is associated with poor survival and increased metastatic activity, and when genetically overexpressed in the mammary epithelium is sufficient to induce mammary tumorigenesis. Additionally, phospho-Tyr211 PCNA can be induced through c-Abl via Ron signaling. Thus, we hypothesize ablation of phospho-Tyr211 within the context of Ron overexpressing breast cancer diminishes tumorigenic potential and impairs metastatic aggression relative to wild-type PCNA counterparts. Using a “knock-in” PCNAY211F mouse model, which ablates phospho-Y211 PCNA formation, crossed with mice with mammary epithelial overexpression of Ron (MMTV-Ron), we show delayed tumor initiation and reduced metastatic aggression in the absence of Tyr211 phosphorylation of PCNA. These data support the previously elucidated mechanism in which Ron signaling through c-Abl induces phospho-Tyr211 PCNA which leads to proliferation of breast cancer cells, and validates the presence of this molecular process in vivo in a clinically relevant breast cancer model.

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The Ron Receptor Promotes Tumor Growth Through Inhibition of IRAK4 and Suppression of Type I Interferon Signaling

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Breast cancer (BC) is still a major killer of women in the US and the development of novel treatments to combat BC is urgently needed. The Ron receptor is overexpressed in human BC and predicts poor outcomes, but the mechanism by which Ron promotes aggressive BC is unknown. To identify potential mechanisms, RNA Seq analysis and validation was performed on Ron modulated BC cells. The most dramatically altered networks involved suppression of Toll/Interleukin-1 receptor (TIR) pathways. We hypothesized that Ron represses TIR signaling in BC cells required for autocrine activation and immune cell recruitment. Knockdown of Ron signaling in BC cells implanted into orthotopic tumor models significantly impairs tumor establishment as well as growth in immunocompetent (IC) mice. When implanted into immunodeficient (ID) mice, tumor establishment is restored but growth is still inhibited. These studies show that tumor cell intrinsic Ron expression promotes cell growth and suppresses immune responses. We also discovered a direct interaction between Ron and IRAK4, which appears to inhibit IRAK4 mediated TIR signaling. When IRAK4 was overexpressed in BC cells, TIR repression was overcome and allowed for immune rejection in IC and decreased tumor growth in ID mice. These data suggest that combination therapies using Ron inhibitors and immunomodulators may provide therapeutic benefit for BC patients.

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The Role of FAK in Tumor Microenvironment

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Focal Adhesion Kinase (FAK) is a 125-kDa cytoplasmic non-receptor tyrosine kinase important in mediating signal transduction in cell migration, survival and proliferation. Up-regulation of FAK in the tumor cells has been shown to enhance tumor malignancy and correlate with poor prognosis. FAK signaling has also been implicated in impacting tumor microenvironment, including endothelial cells and fibroblasts. However, much less is known about how FAK in the tumor stroma affects tumor progression, or how stromal FAK cross-talks with cancer cells. Here we use an inducible Col1a2-creERT, FAK f/f genetic engineered mouse model (GEMM) to specifically delete FAK in the fibroblasts. Using the GEMM, we found that FAK in stromal fibroblasts facilitates breast tumor growth and metastasis in vivo. We further showed that conditioned media (CM) from human breast cancer cells induced FAK Y397 autophoslphorylation and expression of smooth muscle actin (SMA, a marker for cancer-associated-fibroblasts [CAFs]) of normal human lung fibroblasts. Interestingly, only the breast cancer CM educated fibroblasts, but not the naive un-treated fibroblasts, were able to promote breast cancer cell migration and invasion. Lastly, knockdown of FAK in these “educated” fibroblasts abolished their ability to promote breast cancer cell migration and invasion. Taken together, these data suggest a potential role of stromal FAK in creating a pro-tumorigenic tumor microenvironment for breast cancer development and progression, and provide further rationale in therapeutic benefits of FAK inhibition against various solid tumors.

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Suppressing Growth and Metastasis of Hormone-dependent Human Breast Cancer Using a Combination of p53-reactivating Compound APR-246 and a Phosphatidylserine-targeting Antibody

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Almost 70% of all human breast cancers are hormone-dependent and are initially treated with anti-hormone therapy. However, these tumors frequently become drug-resistant and metastasize, leading to patient death. It is therefore vital to develop novel therapeutic strategies to prevent metastasis. Many hormone responsive breast tumors express mutant tumor suppressor p53 gene. Mutant p53 protein (mtp53) lacks the ability to promote apoptosis or control angiogenesis. Consequently, restoration of p53 function is a promising therapeutic strategy. We determined whether administration of APR-246, a small molecule that restores p53 function, together with 2aG4, an antibody that targets phosphatidylserine exposed in the tumor microenvironment, including on tumor blood vessels, effectively suppresses advanced hormone-responsive breast cancer. Human T47-D and anti-hormone resistant BT-474 cells, both of which express mtp53, were injected into nude mice. When xenografts reached 100 mm^3, 3 mice were treated with APR-246 (50-100 mg/kg iv/3 x week) ± 2aG4 (100 mg/kg ip/3 x week). We observed that (1) tumor growth was more effectively suppressed by combination treatment than either agent alone, (2) induction of cell death was significantly higher in combination treatment compared with either agent alone (TUNEL assay), (3) combination therapy eradicated some tumors, and (4) combination therapy reduced metastasis to lymph nodes. We propose that by simultaneously targeting mtp53 protein and phosphatidylserine we may improve control of primary and drug-resistant breast tumor growth and metastasis in breast cancer patients. Support (i) Aprea AB, Sweden, and (ii) generous donors of Ellis Fischel Cancer Center, and a Faculty research grant, University of Missouri, Columbia.

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Bisphenol A Induces Sox2 in ER+ Breast Cancer Stem Cells

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UTHSC

Bisphenol A (BPA) is an endocrine disrupting compound used in food and beverage plastic containers that has been shown to increase breast cancer cellular proliferation. However, the concentrations of BPA used in these experiments are far higher than the physiological levels of BPA detected in the human body. We observed in vitro that exposure of MCF-7 cells to physiological concentrations of BPA failed to increase cell proliferation or to induce canonical estrogen-responsive genes (pS2 and progesterone receptor, PR), in contrast to 17b-estradiol (E2) treatment. However, MCF-7 cells treated with 10 nM BPA induced ALDH1 expression, a marker of human mammary stem cells. When treated with 10 nM BPA, mammospheres derived either from MCF-7 cells, a patient-derived xenograft or the normal mouse mammary gland, exhibited increased size; however, these effects were not observed for MDA-MB-231 mammospheres. Mechanistically, BPA induced SOX2 mRNA and protein in MCF7 mammospheres, resulting from enhanced CREB phosphorylation, and subsequent binding of pCREB to a SOX2 downstream enhancer. These findings suggest that physiological levels of BPA increase ER+ breast cancer tumor maintenance through enhanced cancer stem-like cell activity via direct regulation of SOX2 transcription.

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Inhibition of Autophagy Sensitizes BRCA1-deficient Breast Cancers to the Mitochondrial Inhibitor Metformin

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Breast cancer is a heterogeneous disease and the stratification of patients based on specific disease subtypes is fundamental to the success of treatment modalities. Breast tumors deficient in BRCA1 are mostly associated with basal-like breast cancers and targeted therapeutics for this disease subtype are still lacking. Previously, we showed that inhibition of the essential autophagy gene, FIP200, limits the tumorigenesis and progression of MMTV-PyMT mammary tumors. To address whether autophagy inhibition will be effective in BRCA1-deficient mammary tumors, we generated mice with conditional deletion of FIP200 along with BRCA1 and P53, through utilization of the K14-Cre transgene. Similar to our results in the MMTV-PyMT model, deletion of FIP200 delayed tumorigenesis in the BRCA1-deficient model when compared to wild type and heterozygous FIP200 controls. However, tumor growth and the distribution of histological subtypes were not affected by loss of FIP200. Interestingly, loss of FIP200 decreased mitochondrial mass and oxidative respiratory capacity of BRCA1-deficient tumor cells. These phenotypes which are due to FIP200 deletion correlated with a decrease in the phosphorylation of mTOR substrates and transcript levels of genes involved in mitochondrial biogenesis. Importantly, we observe an increased sensitivity to mitochondrial disrupting agents upon loss of FIP200. Consequently, we showed that the combination of the autophagy inhibitor, Spautin-1, along with the mitochondrial Complex I inhibitor, Metformin, was more effective in reducing oxidative respiratory capacity and colony forming ability of BRCA1-deficient breast cancer cells. Altogether, our results indicate that inhibition of autophagy may increase the benefits of metformin treatment in BRCA1-deficient breast cancers.

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The Mechanisms of Improved Effectiveness of Combined Targeting of ERa and XPO1

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There is a critical need for novel therapeutic approaches to resensitize recurrent ERa (+) tumors to endocrine therapies. The objective of this study was to elucidate mechanisms of improved effectiveness of combined targeting of ERa and XPO1, a nuclear transport protein in overcoming endocrine resistance. Using Cignalfinder pathway profiling, Seahorse metabolic profiling and GC/MS whole metabolite profiling, we found that combination of 4-OH-Tam and Selinexor (SXR), a highly selective XPO1 inhibitor that is currently in clinical trials for leukemias and prostate cancer, inhibited Akt phosphorylation by changing the localization of the kinase. Since we observed dramatic changes in Akt activity we hypothesized that glucose utilization pathways and consequently metabolic profile of breast cancer cells would change in the presence of 4-OH-Tam and SXR. Their glucose and fatty acid dependency decreased in the presence of SXR and cells were more dependent on glutamine as the mitochondrial fuel source. In order to examine metabolites that might result in the observed phenotype we performed whole metabolite profiling and identified proline metabolic pathways to be upregulated when cells were treated with SXR+4-OH-Tam. We demonstrated that combined targeting of XPO1 and ERa rewires metabolic pathways, increase demand on mitochondria and causes increased production of ROS that would eventually lead to apoptosis. Remodelling metabolic pathways to regenerate new vulnerabilities in endocrine resistant tumors is novel, and given the need for better strategies for improving therapy response of relapsed ERa(+) tumors, our findings show great promise for uncovering the role ERa-XPO1 crosstalk plays in reducing cancer recurrences.

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Normal and Perturbed Phospho-signaling of Granulocyte Colony Stimulating Factor Receptor (G-CSFR) in Myeloid Cell Development, Myeloid Leukemia and Neutrophilic Leukemia

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Granulocyte-colony stimulating factor receptor (G-CSFR) signaling plays a seminal role in neutrophil production through activation by its ligand, granulocyte colony stimulating factor (G-CSF). A series of CSF3R mutations have been reported to cause neutropenia and/or leukemia, and can be classified as either proximal point or distal truncation mutations based upon where they occur in G-CSFR. A proximal point mutation, T618I, has been reported in >80% of chronic neutrophilic leukemia (CNL) patients. Several distal truncation mutations of CSF3R are recorded in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patient groups progressed from neutropenic condition. It is not clear how these two types of the mutated receptor signal differently compare to the normal receptor leading to the leukemic condition. To study this, we developed an in vitro model system using virally transduced BaF3 cells expressing WT, proximal mutation T618I, and distal truncation mutation Q741x. The model was validated using immunoblots and phospho-kinase array for STAT3/5 after G-CSF activation. To study phospho-signaling cascades, SILAC-based quantitative phosphoproteomics studies were performed with WT and mutant receptors by sequential enrichment for pTyr with pY1000 antibodies and pSer/pThr on Titanium dioxide followed by nanoLC-MS/MS analyses. We have identified over 12,000 unique phosphorylation sites with quantitative changes in 60-70 pTyr sites and over 1,600 pSer/pThr sites in response to G-CSF stimulation and/or in WT versus mutant cell types. Our phosphoproteomics finding showed an upregulation of phospho-sites for the proteins involved in the receptor recycling/degradation mechanism by WT but not for the mutant G-CSF receptors.

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Eradication of Acute Myeloid Leukemia with FLT3 Ligand-targeted miR-150 Nanoparticles

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Acute myeloid leukemia (AML) is a common and fatal form of hematopoietic malignancy. Overexpression and/or mutations of FLT3 have been shown to occur in the majority cases of AML. Our analysis of a large-scale AML patient cohort (n=562) indicates that FLT3 is particularly highly expressed in some subtypes of AML such as AML with t(11q23)/MLL-rearrangements or FLT3-ITD. Such AML subtypes are known to be associated with unfavorable prognosis. To treat FLT3-overexpressing AML, we developed a novel targeted nanoparticle system: FLT3 ligand (FLT3L)-conjugated G7 poly(amidoamine) (PAMAM) nanosized dendriplex encapsulating miR-150, a pivotal tumor-suppressor and negative regulator of FLT3. We show that the FLT3L-guided miR-150 nanoparticles selectively and efficiently target FLT3-overexpressing AML cells, and significantly inhibit viability/growth and promote apoptosis of the AML cells. Our proof-of-concept animal model studies demonstrate that the FLT3L-guided miR-150 nanoparticles tend to concentrate in bone marrow, and significantly inhibit progression of FLT3-overexpressing AML in vivo, while exhibiting no obvious side effects on normal hematopoiesis. Collectively, we have developed a novel targeted therapeutic strategy, using FLT3L-guided miR-150-based nanoparticles, to treat FLT3-overexpressing AML with high efficacy and minimal side effects.

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Uncover TET1 Targets in MLL-rearranged Leukemia

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The expression of Ten-Eleven-Translocation 1 (TET) protein and the global level of its enzymatic product, 5hmC, is markedly reduced in a wide range of tumors, including melanoma, prostate, breast, lung, and liver cancer, suggesting a tumor suppressive role of TET1. However, our recent study demonstrated that TET1 expression and the associated 5hmC levels are significantly up-regulated in MLL-rearranged leukemia, revealing an oncogenic role of TET1 in this type of acute myeloid leukemia (AML). Nonetheless, how TET1, as a methylcytosine dioxygenase, plays its oncogenic role in AML is still unclear.To address this issue, we performed stable isotope labeling by amino acids in cell culture (SILAC)-based proteomic profiling to systematically explore the functional targets of TET1 in a genome-wide and unbiased way. When TET1 was knocked down in MLL-ENL-estrogen receptor inducible (ERtm) mouse myeloid leukemia cells, 123 proteins were downregulated whereas 191 were upregulated with a fold-change cutoff of 1.2. After taking into account the correlation of TET1 and its candidate targets in several sets of AML patient samples, we focused on IDH1 and PSIP1, which represent the negatively- and positively-regulated targets of TET1, respectively. Chromatin immunoprecipitation (ChIP) assays suggest that TET1 directly binds to the CpG islands in the promoters of these two genes. Forced expression of Idh1 in bone marrow cells of MLL-AF9 leukemic mice significantly inhibited the colony-forming capacity, which mimics the effect of TET1 knock-out. Considering the important roles of IDH1 and PSIP1 in AML, our findings may lead to the development of targeted therapy of AML.

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Ron Receptor Tyrosine Kinase Mediates Intricate Communication Between Tumor Cells and Macrophages in Breast Cancer

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Breast cancer (BC) is one of the leading causes of cancer related death in women worldwide. Though the localized disease is potentially curable, metastatic and recurrent disease carries a dismal prognosis. Of late, the tumor microenvironment in breast cancer is recognized as a critical participant in tumor progression and therapeutic responses. Recently we identified a novel function for Ron tyrosine kinase receptor in the tumor microenvironment demonstrating Ron loss in myeloid cells, specifically macrophages reduced tumor growth and ablated metastasis in established murine models of metastatic BC. Using co-culture systems for mechanistic analyses, we further demonstrate that loss of Ron in macrophages induces apoptosis in BC cells accompanied by growth reduction. Interestingly, we observed a robust chemotactic response between Ron expressing BC cells and normal macrophages. Conversely, Ron knockdown in BC cells impairs migration towards macrophages. Similar chemotactic response was observed with conditioned media obtained from BC cells and macrophages and we further show that this is mechanistically dependent upon Ron signaling for the induction of secreted chemoattractants. Collectively, our findings show that the Ron receptor directs the interplay between epithelial cells and macrophages facilitating BC cell growth and migration. Our future studies will provide insight into the role of Ron receptor in subverting the normal chemokine system to a exert tumor promoting role in breast cancer.

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Different Binding Modes of Human CST Mediate Specific Aspects of Telomere Replication and Genome-wide Replication Rescue

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Human CST (CTC1-STN1-TEN1) is an ssDNA-binding complex that was originally identified as a DNA polymerase α stimulatory factor. CST functions in telomere replication first by aiding passage of the replication machinery through the telomere duplex and then enabling fill-in synthesis of the telomeric C-strand following telomerase action. CST also has genome wide roles in the resolution of replication stress. CST bears striking resemblance to RPA, the ssDNA binding protein responsible for moderating key transactions in DNA replication, recombination and repair. STN1 and TEN1 contain OB fold domains and are structurally similar to RPA2 and RPA3 respectively. While CTC1 is much larger than RPA1, the C-terminus is predicted to harbor three OB folds with high structural similarity to the three DNA binding motifs of RPA1 (OB folds A-C). The similarities between CST and RPA suggested that the various functions of CST might utilize subsets of OB folds for different modes of DNA binding. To address this possibility, we generated a CST DNA binding mutant by altering three residues in the STN1 OB fold (STN1-OBM). The equivalent residues in RPA2 contact or lie close to DNA in the crystal structure. *In vitro* studies indicated that STN1-OBM moderately decreases CST binding to short G-strand oligonucleotides; however binding to long telomeric or non-telomeric oligonucleotides is largely unaffected. These results indicate that the STN1 OB fold is responsible for high affinity binding to short stretches of telomeric G-strand DNA. Moreover, CST appears to resemble RPA in exhibiting different DNA binding modes but the trajectory of DNA engagement is different. To determine the *in vivo* effect of altered DNA binding, we asked if STN1-OBM expression alters telomere replication or genome-wide replication rescue. Interestingly, we found STN1-OBM to be a separation of function mutant. The STN1-OBM cells had increased anaphase bridges and multiple telomeric FISH signals (MTS). However, the length of the telomeric G-overhang and the rate of C-strand fill-in were normal. Likewise, the cells showed wild type sensitivity to hydroxyurea (HU) and the level of new origin firing after release from HU was unaffected. Thus, the ability to bind short stretches of ssDNA appears to be important for replication through natural barriers such as telomeres but is less critical for C-strand fill-in or stress-induced origin firing. Overall our work suggests that CST binds DNA dynamically via multiple OB folds and mediates different transactions via specific DNA binding modes.

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Tumor Cell Autonomous and Non-Autonomous Functions of the Ron Receptor in Prostate Tumorigenesis

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Metastatic prostate cancer is a leading cause of cancer-related death among men in the United States. The Ron receptor tyrosine kinase is overexpressed in prostate cancer, and Ron expression increases with disease severity in humans and mouse models. These findings suggest that Ron drives progression to advanced disease. Prior studies in our laboratory showed that complete loss of Ron in the TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) spontaneous prostate cancer model reduced tumor growth, angiogenesis and tumor cell survival. Orthotopic transplantation studies from our laboratory have shown that Ron signaling specifically in two separate cell types promotes tumorigenesis: prostate epithelial cells and cells of the myeloid lineage (macrophages/granulocytes). We, and others, have also found Ron to play important indirect roles in promoting tumorigenesis via regulating the tumor microenvironment. Here, we propose to investigate the contributions of Ron expression in the prostate epithelium and in myeloid cells towards prostate tumor development and progression using a single autochthonousTRAMP model. Mice were generated to have conditional loss of Ron specifically within either cell type. To further explore cell type-specific mechanisms, we will compare tumor growth, vascularization, immune cell infiltration and activation status, and metastasis. We hypothesize that the Ron receptor mediates cell intrinsic and extrinsic signaling in both the prostate epithelium and myeloid cells to regulate prostate tumor growth and progression. This work will provide insight into important functions of cell type-specific Ron signaling in prostate tumorigenesis, which could impact therapeutic strategies for treatment of prostate cancer.

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MiR-200b Targets Actin Cytoskeleton Regulators and Inhibits Triple Negative Breast Cancer Metastasis

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Triple negative breast cancer (TNBC) accounts for 10-20% of newly diagnosed breast cancer cases. Compared to other subtypes of breast cancer, TNBCs usually show more aggressive metastasis. Unfortunately, the mechanism of TNBC metastatic behavior is not clear. MiR-200 family members are among the first miRNAs reported to function as potent inhibitors of epithelial to mesenchymal transition, an important event in cancer metastasis. However, the effect of miR-200 family on TNBC metastasis is not well understood. In this study, we investigated the effect of miR-200b, one member of the miR-200 family, on TNBC metastasis using cell culture and mouse orthotopic mammary xenograft tumor models. We found that the expression level of miR-200b is significantly lower in TNBC cells and tissues than that in other types of breast cancer. Stably expressing miR-200b significantly reduced TNBC cell migration, invasion and tumor metastasis. Mechanistic studies revealed that miR-200b overexpression in TNBC cells caused drastic changes in cellular actin cytoskeleton organization patterns as evidenced by reduced lamellipodia formation but increased stress fiber formation. In consistent with these findings, Rho GTPase pulldown assays demonstrated that stably expressing miR-200b significantly increased Rho A activation, but reduced Rac1 activation. Moreover, inhibition of Rho A signaling impaired the inhibitory effect of miR-200b on TNBC cell migration and tumor metastasis. Further bioinformatics analysis and experimental studies identified some actin cytoskeleton regulators as key targets of miR-200b. Together, these findings suggest that miR-200b inhibits TNBC metastasis by targeting actin cytoskeleton regulators, which play important roles in promoting TNBC metastasis.

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Impact of Ethnicity-dependent Differences in Normal and Tumor Epigenome on Breast Cancer Progression

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African American women suffer higher mortality from the aggressive breast cancer subtype, triple negative breast cancer (TNBC), than Caucasian women. Furthermore, basal-like and mesenchymal stem-like TNBCs with elevated intra-tumor heterogeneity are significantly more common in African American women. In contrast, breast cancer in Hispanic women is generally less aggressive. Whether worse outcome in African American women is due to an increased incidence of TNBC or unique biological factors that promote aggressive biology is an important but unresolved challenge in cancer disparities research. To test whether TNBCs in African American women are a mutation-driven, molecularly distinct disease or have inherently aggressive biology driven by disparities, we carried out an extensive phenotypic, epigenomic, and transcriptomic characterization of normal breast epithelial cells samples of African American, Caucasian, and Hispanic women. By using a unique culturing system for propagating normal epithelial cells of different lineages, including differentiated-luminal, luminal-progenitor, and stem cells from the breast core biopsies of healthy African American, Caucasian, and Hispanic women, we demonstrated selective enrichment of multi-potent progenitor cells in normal African American breast. We showed a gene expression pattern overlapping mesenchymal-like subtype TNBC, indicating that African American TNBCs originated from a distinct population of breast epithelial cells with inherent mesenchyme-like (cancer-prone) features. Epigenetic profiling of cancer-prone luminal progenitor cells and less-cancer prone differentiated cells revealed striking ethnicity-dependent differences in DNA methylation and gene expression affecting cellular differentiation and response to stemness and de/differentiation-associated microenvironmental cues. Cells typically considered to be differentiated, based on the cell surface marker profile CD49f-/EpCAM+, showed remarkable ethnic differences in DNA methylome and transcriptome. For example, presumably differentiated cells in African American women retained expression of several stem/progenitor cell-enriched genes such as ZEB2 but had lower GATA3 expression, a key component of the master cell-type specific transcription factor network linked to luminal progenitor differentiation and response to steroid hormone estradiol. In addition, pathways associated with tight junction signaling and HIPPO signaling, both required for pregnancy-associated differentiation, were hypermethylated only in CD49f- /EpCAM+ cells of African American women. Cells isolated from healthy Hispanic women breast lacked those features and moreover displayed differentiated (less-cancer prone) characteristics, such as extensive methylation of pluripotency-associated genes and Wnt/ß-catenin signaling in luminal progenitor cells. In summary, our integrated DNA methylome and transcriptome studies of purified subpopulation of cells showed that phenotypically differentiated cells of African American women express genes associated stemness, cancer-prone luminal progenitors, and plasticity compared to similar cells from Caucasian and Hispanic women. We suggest that differences in normal breast epithelial cell hierarchy of ethnic groups may impact tumor evolution/ heterogeneity, and ethnic differences exist in differentiation of stem to mature cells. In addition, identifying ethnicity-dependent markers of breast cancer risk may call for reassessment of clinically used prognostic/predictive markers that consider ethnicity as a confounding factor.

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A Re-wired Metabolic Circuitry Fuels Cancer Development: Implications for Diagnosis and Targeted Therapy

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In stark contrast to normal cells that only divide a finite number of times, cancerous cells proliferate indefinitely. In order to fuel this bioenergetically and biosynthetically demanding process, oncogenic pathways hijack cellular metabolism to increase the uptake of available nutrients while simultaneously maximizing the anabolic potential of those nutrients. We have previously uncovered a novel mechanism critical for cancer development whereby oncogenic signaling coordinates the proportional increases in protein synthesis and nucleotide metabolism, via the regulation of a single rate-limiting enzyme, phosphoribosyl-pyrophosphate synthetase 2 (PRPS2). We have developed new genetic, pharmacologic, and chemical tools to exploit this specific dependency of cancer cells on PRPS2-mediated nucleotide production, thus laying the groundwork for innovative approaches to better diagnose and treat patients with cancers that are currently “undruggable”. In this regard, we have generated PRPS2 knockout mice, which exhibit no obvious phenotypic differences when compared to wild-type counterparts, yet display a dramatic reduction in tumor formation when crossed to various genetically-engineered mouse models of cancer. Additionally, in collaboration with Michael Evans at UCSF, we have designed a novel PRPS2 radiosubstrate mimetic capable of non-invasively imaging cancers in vivo. In conclusion, we have unraveled a novel feed-forward anabolic circuit that fuels cancer development upon a variety of oncogenic lesions. Moreover, we have built an entire suite of tools that will allow us to decipher the role of nucleotide metabolism during normal development and disease, therefore resulting in unprecedented biological insights as well as marked improvements in diagnosis and treatment of patients with cancer.

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Pro-versus Anti-tumorigenic Role of Estrogen Receptor-beta in Breast Cancer is Determined by p53 Status

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Whether estrogen receptor beta (ERß) is an oncogenic or a tumor suppressor protein in breast cancer has been controversial. ERß levels are high in ERa negative cancers including triple-negative breast cancer (TNBCs). Recent reports including the Cancer Genome Atlas (TCGA) show that about 80% of TNBC express mutant p53 (mut-p53) and it is the most predominant driver in these cancers. We tested the hypothesis that p53 status in breast cancer will have an important role in determining function of ERß. We report that ERß directly binds to p53 in human breast cancer cells and have mapped the domains of interaction. Using the highly sensitive proximity ligation assay (PLA), we show ERß-p53 interaction in situ in breast cancer cells and TNBC tissues expressing either wt- or mut-p53. Surprisingly, we found that ERß has opposite functions depending on the wt/mut status of p53. In the context of the wt-p53, ERß is pro-proliferative, whereas in the context of mut-p53, ERß is anti-proliferative. ERß binds and sequesters mut-p53 from mut-p53-p73 complex leading to reactivation of tumor suppressor p73. Combination of immunohistochemistry (IHC) and PLA in TNBC patient tissue microarray (TMA) followed by correlative analysis of linked database of patient tumor characteristics and disease outcome showed that p53 status is an important determinant of pro- versus anti-tumorigenic role of ERß in these tumors. These data would have significant clinical implications in targeting ERß and mutant p53 signaling pathways for diagnostic, prognostic, and therapeutic purposes especially in ERa negative cancers such as TNBC.

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27-hydroxycholesterol an ER and LXR Modulator Increases Breast Cancer Metastasis Through Its Actions on the Host

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Breast cancer remains the second leading cause of cancer death in women. Associated mortality is most often due to the metastatic spread of breast cancer, highlighting the need for novel approaches to prevent and treat this stage of disease. In this regard, both obesity and elevated cholesterol have been associated with a decreased relapse free survival time. Furthermore, patients taking inhibitors of HMGCoA-reductase (HMGCR, statins) experience a significantly increased relapse free survival time. Therefore, we tested the hypothesis that cholesterol promotes the metastasis of breast cancer. Since we have shown that a primary metabolite of cholesterol, 27-Hydroxycholesterol (27HC), exerts partial agonist activity on both the estrogen receptors (ERs) and liver X receptors (LXRs), we determined whether 27HC may mediate the potential metastatic effects of cholesterol. Interestingly, we demonstrate that 27HC does increase metastasis to the lung, but its activities require the presence of host immune cells. Indeed, we find that 27HC engages immune cells to alter the metastatic microenvironment in such a way that facilitates cancer cell colonization and growth. Our ongoing work is aimed at identifying the specific mechanisms by which 27HC induces these changes and thereby increases metastasis. In summary, our data strongly suggests that 27HC is the biochemical mediator of the effects of cholesterol on breast cancer metastasis, providing additional support for the exploration of lower cholesterol diets, pharmacological inhibitors of either HMGCR or the enzyme responsible for the synthesis of 27HC (CYP27A1), and immune cell targeting strategies in the treatment of metastatic breast cancer.

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Progestins Induce Stem-Cell Like Properties in Hormone-dependent Human Breast Cancer Cells

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Clinical trials show that post-menopausal women undergoing combined hormone replacement therapy containing estrogen (E) and progestins (P) have an increased risk of breast cancer compared with women taking E alone. We showed that both natural and synthetic P accelerate the development of breast tumors *in vivo* and increase lymph node metastasis, leading us to hypothesize that P-induced tumor growth and metastasis may be mediated by an enrichment of the cancer stem cell (CSC) pool. With this in mind we used flow cytometry to examine whether P influences the expression of CSC markers (CD44 and ALDH) in breast cancer cells and found that natural P, as well as a variety of synthetic P (10 nM) increased CD44 levels in both T47-D and BT-474 cells. Induction of CD44 was blocked by the antiprogestin RU-486, demonstrating that induction of CD44 is progesterone receptor dependent. Other steroid hormones had no effect on CD44 expression. A subset of the induced $CD44^{high}$ cells demonstrated high ALDH enzyme activity. The synthetic progestins medroxyprogesterone acetate and norethindrone significantly increased mammosphere formation, suggesting that enrichment of the $CD44^{high}$ and $ALDH^{bright}$ subpopulation of cancer cells is of functional significance. Based on our observations we contend that exposure of breast cancer cells to synthetic P may lead to an enrichment of the CSC-like pool, supporting the development of P-accelerated tumors *in vivo*. Supported by generous gifts from donors of Ellis Fischel Cancer Center, and by a Faculty award from the College of Veterinary Medicine, University of Missouri, Columbia.

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Integrative –omics Approach Identifies Novel Roles for Extra-nuclear ERa Signaling in Rewiring Cancer Cell Metabolism During Obesity-associated Postmenopausal Breast Cancer

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Obesity is a preventable risk factor for post-menopausal ERa(+) breast cancer. We hypothesized that serum from obese post-menopausal women contain factors that would increase tumorigenicity of breast cancer cells. Using whole metabolite profiling and OLINK biomarker panel of about 400 proteins associated with cancer, inflammation and cardiovascular disease, we identified biomarkers that were differentially present in serum from 100 obese and non-obese postmenopausal women. Next, using in vitro cell based assays as proxy we identified free fatty acids (FFAs) as factors from serum that correlate with increased cell proliferation, motility and mTOR activation in ERa(+) breast cancer cells. We performed RNA-Seq, ERa ChIP-Seq and metabolomics analysis in breast cancer cells that are exposed to conditions that mimic serum from obese postmenopausal women. This integrative –omics approach enabled us to uncover ERa and mTOR pathway-dependent metabolic rewiring in breast cancer cells under these conditions. Pathway preferential estrogens (PaPEs), which target ERa and mTOR signaling, were able to block free fatty acid-dependent proliferation of breast cancer cells. In fact, efficient cancer cell killing by PaPEs was achieved only in the presence of FFAs, suggesting a role for obesity-associated metabolic rewiring in providing new vulnerabilities for the breast cancer cells. In summary, we uncovered a novel role for extranuclear-initiated ERa signaling in rewiring breast cancer cell metabolism in response to obesity-associated factors in the serum. Our findings provide a basis for preventing or inhibiting obesity-associated breast cancer by using PaPEs that would exploit new metabolic vulnerabilities of breast tumors in obese postmenopausal women.

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Interplay between CTC1 and POT1 prevents telomeric DNA damage and damage signaling

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Human CST (CTC1-STN1-TEN1) functions in telomere duplex replication, C-strand fill in, and genome-wide replication rescue in response to replication stress. Mutations in the CTC1 subunit cause the disease Coats plus and can lead to dyskeratosis congenita. However, the specific role of CTC1 in telomere maintenance is not well understood. In order to study the function of human CTC1, we generated a conditional gene disruption in HCT116 cells, a human colon cancer cell line. The cells exhibit a gradual decrease in growth rate starting around day 7 after knock-out induction. By three weeks, the cells cease to divide and undergo senescence. The phenotypes related to telomere dysfunction become clearly apparent from day 7 onwards. These phenotypes include increased G-overhang abundance and heterogeneity in telomere length, reflecting abnormal telomere growth and telomere shortening. We also observe sister chromatid associations and some telomere loss but we do not see a significant increase in chromosome fusions. Strikingly, γH2AX is observed at telomeres that retain telomeric DNA/FISH signals. However, γH2AX generally does not co-localize with chromosome ends lacking telomeric FISH signals (i.e. signal free ends) indicating that these chromosome ends likely retain some telomeric DNA. The effect of human CTC1 disruption differs from what was observed in mouse cells where CTC1 disruption caused massive telomere loss and a strong DNA damage response at the signal free ends. Moreover, γH2AX was not detected at telomeres that retained telomeric DNA. Interestingly, the DNA damage signaling and sister chromatid associations caused by loss of human CTC1 are partially rescued by overexpressing the shelterin component POT1. These data suggest that the senescence caused by CTC loss may be due to prolonged DNA damage signaling.

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Therapeutic Targeting of Cyclic GMP Signaling in Head and Neck Cancer

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Objectives: Common treatments for head and neck squamous cell carcinoma (HNSCC) often result in failure and adverse side effects, providing major incentives to develop alternative therapies. Activation of soluble guanylate cyclase (sGC) leads to increased cyclic GMP (cGMP), which is broken down by phosphodiesterase 5 (PDE5). The role of the cGMP pathway in the pathophysiology of HNSCC, especially in terms of induction of apoptosis, has not been investigated. Our objectives were: 1) to examine the effects of sGC activators or PDE5 inhibitors on the viability of HNSCC cells, 2) to determine whether they increase the efficacy of chemotherapy, targeted therapy, and/or ionizing radiation, and 3) to determine whether they suppress xenograft growth in mice.

Results: Treatment of HNSCC cell lines with sGC activators (YC-1 or BAY 41-2272) or a PDE5 inhibitor (Cialis) decreased cell viability and induced apoptosis. Co-treatment at low drug doses enhanced the suppressive effects of Cisplatin, 5-Fluorouracil, Lapatinib (EGFR inhibitor), and ionizing radiation on cell viability. CAL27 cells were inoculated sc in the flank of athymic *nude/nude* female mice. A continuous delivery of Cialis via Alzet osmotic mini-pumps caused marked suppression of tumor growth.

Conclusions: cGMP pathway activation induces apoptosis and enhances the efficacy of chemotherapy, targeted therapy, and ionizing radiation in HNSCC cells. PDE5 inhibitors such as Cialis are FDA-approved to treat erectile dysfunction, while a sGC activator, Riociguat, is approved for treating pulmonary hypertension. Analysis of tumor sGC/PDE5 activity should be predictive of response to treatment with the above drugs in head and neck cancer.

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Evaluating S6K as a Target in Breast Cancer

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Amplification of the ribosomal protein S6 kinase (S6K) proteins has been noted in almost 15% of breast cancer tumors. We hypothesize that functions of S6K include driving cell cycle progression and resistance to apoptosis. Using pharmacologic and genetic approaches we inactivated these kinases in breast cancer cell lines that have amplified S6K expression. Data show that inhibition of S6K reduces substrate phosphorylation in cells in all phases of the cell cycle. This inactivation also reduced the progression of cells into S phase. The effect of S6K inhibition on cell cycle progression was enhanced when used in combination with Fulvestrant or Lapatinib, suggesting cooperative function in chemotherapy. These results suggest that in patients with S6K amplification, the pharmacologic inhibition of S6K, which is generally well tolerated, could increase the efficacy of current therapies when used in concert.

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Single Dose and Steady State Brain and Plasma Pharmacokinetics of Letrozole in Female Sprague Dawley Rats

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Anecdotal reports have indicated that letrozole, a potent aromatase (CYP19A1) inhibitor, may be effective in treating brain metastases from breast cancer but our current knowledge about its extent of brain penetration and disposition is limited. Here, we comprehensively evaluated plasma and brain extracellular fluid (ECF) pharmacokinetics of letrozole in female Sprague Dawley (SD) rats following single and multiple doses.For this study, we employed adult female (201-225g; N=13) jugular-vein cannulated SD rats for serial blood sampling. Letrozole (4mg/kg) was administered intraperitoneally and intracerebral microdialysis was employed to analyze its unbound levels in the brain ECF. Plasma and ECF concentrations were estimated by a HPLC method and the data were analyzed using Phoenix WinNonlin.Essentially, following i.p. administration of letrozole, the terminal half-life was 36± 2h, resulting in plasma AUC of 18± 3 h*µg/ml and ECF AUC of 5± 1 h*µg/ml. The accumulation indices ranged from 2.7– 4.0 reflecting marked letrozole accumulation following multiple dosing with steady state plasma and brain ECF values of 51± 3 and 24± 2 h*µg/ml respectively. Furthermore, brain-to-plasma ratios (indicative of CNS penetration) ranged from 0.8–1.2.Our study indicates that letrozole easily penetrates the blood–brain barrier in female SD rats and that therapeutically relevant concentrations can be attained in the brain upon chronic dosing. This finding further supports the clinical implication for use of this compound for treatment of brain metastasis of: i) ER positive breast cancer patients previously untreated with letrozole; ii) anti-estrogen resistant breast cancer patients who have undergone therapy with HDAC inhibitors.

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Phosphorylated eIF4E is a Molecular Driver of All Subtypes of Breast Cancer in Women

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A better understanding of the molecular drivers of breast cancer is critical for discovering new breast cancer therapies. Protein synthesis (translation) is essential for cancer cell survival, growth, and proliferation. Targeting translation is potentially a novel strategy for therapy of epithelial cancers. Since eIF4E, a rate limiting step in translation, regulates the translation of multiple malignancy associated mRNAs and is activated by phosphorylation, we hypothesized that phosphorylation of eIF4E has a critical role in driving breast cancer. Using immunohistochemistry and semi-quantitative cellular pathology approaches we analyzed expression of eIF4E and phospho-eIF4E (p-eIF4E) in 168 FFPE archived surgical specimens of normal breast, benign breast disease and pre-invasive, invasive, and metastatic breast cancer. Stain in the cytoplasm and nuclei of breast epithelial cells was evaluated microscopically by the area of epithelium stained and the intensity of stain. Expression of both eIF4E and p-eIF4E in the cytoplasm and nuclei was highly correlated with tumor progression . Interestingly, while expression of both eIF4E and p-eIF4E was not significantly increased in benign breast disease, expression of both eIF4E and p-eIF4E was significantly increased in atypical duct hyperplasia and in carcinoma in situ as well as in invasive ductal carcinoma relative to normal duct epithelium. Breast cancers of all receptor types had increased expression of eIF4E and p-eIF4E suggesting that eIF4E and p-eIF4E are critical in breast cancer development and progression and that targeting either eIF4E or phosphorylation of eIF4E may be an effective strategy for therapy of all subtypes of breast cancer in women.

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Pharmacological Targeting of GTP-energy Sensor, PI5P4Kß, As A Novel Breast Ccancer Therapeutics

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Mutations of the p53 gene, which occur in >30% of breast cancer patients, confer high resistance to chemotherapy and predispose patients for recurrence—the major cause of breast-cancer mortality. There is a desperate need to develop new therapeutic modalities that improve the outcome of p53-mutated breast cancer patients. Previously, we found that PI5P4K (Phosphatidyl-inositol 5-phosphate 4-kinase) is critical for p53-mutated breast cancer growth (Cell, 2014). PI5P4K is an enzyme that converts phosphatidylinositol 5-phosphate (PI5P) to phosphatidylinositol 4,5-phosphate (PI(4,5)P2). The main function of PI5P4Kß is to control these lipid second messenger levels for cell functions. Recently, we have discovered that PI5P4Kß, one of three isoforms of PI5P4K, acts as a cellular sensor for GTP energy levels and regulates cellular metabolism and tumorigenesis (Molecular Cell, 2016). These results suggest that pharmacological inhibition of the PI5P4Kß kinase activity could abrogate tumorigenic activity of p53-mutated breast cancer—revealing a new therapeutic intervention. However, there have been no commercially available PI5P4K inhibitors. Towards this, we have developed a high-throughput drug screening system that consisted of two steps: the first is a crystal structure-based in silico screening, second is a NMR-based inhibitory activity screening. Through these screenings, we have identified a series of unique compounds that inhibit PI5P4Kß activity. One of these compounds turned out to be a FDA-approved NSAID—Non Steroid Anti-Inflammatory Drug. We have co-crystalized the identified compound with PI5P4Kß for further medicinal chemistry. In the symposium, we will introduce this novel screening system and the results obtained from in vitro and in vivo experiments.

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Osteopontin in Breast Cancer Metastasis

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Metastasis formation is an essential aspect of cancer. While the organ preference for dissemination is largely governed by tumor–host interactions on the epigenetic level, there is a genetic basis underlying the ability of cancer cells to disseminate. Metastasis genes are comprised of developmentally non-essential stress response genes, which encode homing receptors, their ligands, and extracellular matrix-degrading proteinases. They jointly cause invasion and anchorage-independence. Metastatic potential is conferred to breast cancer cells by aberrant expression and splicing of the cytokine osteopontin. Its gene product is prone to yielding three RNA messages, osteopontin-a (full length), osteopontin-b (lacking exon 5), and osteopontin-c (lacking exon 4). The shortest form -c is differentially generated in breast cancers, but is absent from healthy tissues. The major limiting factor in the process of diessemination is the death of the tumor cells before their implantation in target organs. Hence, anchorage-independent survival is essential for metastasis formation. While untransformed non-hematopoietic cells undergo anoikis consecutive to losing contact with their substratum, cancer cells can survive in the circulation for extended periods of time. The detachment of mammary epithelial cells prompts a loss of glucose transport and consecutive ATP deficiency, thus compromising the energy metabolism. Invasive breast tumor cells abundantly express the splice variants osteopontin-a and osteopontin-c, which synergize in supporting dissemination via up-regulating the energy production. The elevation of intracellular glucose, mediated by osteopontin-a, and increased ATP generation, mediated by osteopontin-c, synergistically lead to deadherent survival of breast cancer cells. Osteopontin splice variants hold promise as potential drug targets.

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Expression of Genes for Peptide/Protein Hormones and Their Receptors in Breast Carcinomas as Biomarkers Predicting Risk of Recurrence

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Certain peptide/protein hormones in breast cancer appear involved in clinical behavior. We evaluated relationships between genes for these hormones and cognate receptors as independent predictors of risk of recurrence. Expression of genes for 55 peptide/protein hormones (ligands) and 73 cognate receptors were measured by microarray of LCM- procured carcinoma cells from 247 breast biopsies. Total RNA was extracted and expression of 22,000 genes was determined. Univariate and multivariate Cox regressions with interaction were determined. When pairs of hormone ligand and cognate receptor genes were evaluated by multivariate Cox regression with interaction, gene sets predicted risk of recurrence (INS/IGF2R, HAMP/SLC40A1, POMC/MC4R, GH1/GHR and VIP/VIPR2) and OS (CORT/SSTR5, VIP/VIPR2 and GHRH/GHRHR, based on unadjusted p-value for interaction term < 0.05). Since expression *in situ* of both hormone and receptor are necessary to elicit endocrine action, a unique alternative approach using minimum expression values between ligand and receptor for each patient was applied. Results revealed that HAMP/SLC40A1 showed significance in both interaction and minimum models, and was further investigated by splitting ligand and receptor into low (below 1st quartile) and high (above 1st quartile) expression groups. The group consisting of high expression for both ligand and receptor was contrasted with the other three groups (low expression for ligand, receptor, or both). The higher expression group exhibited a better DFS (hazard ratio (HR) = 0.56 95% CI 0.37-0.84, p=0.004) and OS (HR = 0.59 95% CI 0.37-0.93, p=0.022). By determining gene expression directly on pure populations of breast carcinoma cells, we demonstrated many lesions synthesize mRNA species for a wide variety of peptide/protein hormones as well as for their cognate receptors. Using clinical follow-up upto 12 years, univariate and multivariate Cox regression analyses with and without interaction models revealed noteworthy candidates of hormone-receptor complexes that predicted risk of recurrence as well as overall survival. Our results suggests that many breast carcinomas exhibit considerable endocrine autonomy for controlling progression, which warrants investigation of protein products of gene candidates in isolated populations of breast carcinoma cells.

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Smoking History Impacts Gene Expression Levels of Human Breast Carcinoma

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In contrast to studies focused on cigarette smoking and risk of breast cancer occurrence, our emphasis is to explore influence of tobacco smoking on risk of recurrence and progression. Our goal is to combine knowledge of lifestyle behavior (smoking history) and molecular phenotypes of the lesion to improve clinical assessment. Microarray obtained from LCM-procured carcinoma cells from de-identified tissue biopsies identified candidate genes which were validated by qPCR to analyze effects of tobacco smoking on gene expression. Study population consisted of 66 smokers and 99 non-smokers. Using non-parametric methods, distribution of each of the ~22,000 genes represented in the microarray were analyzed by three comparisons: 1) all smokers vs. all non-smokers; 2) smokers with a recurrence vs. those that remained disease-free; and 3) non-smokers with a recurrence vs. those that remained disease-free. Analyses identified 15 genes (APOC1, ARID1B, CTNNBL1, MSX1, UBE2F, IRF2, NCOA1, LECT2, THAP4, RIPK1, AGPAT1, C7orf23, CENPN, CETN1 and YTHDC2) for further investigation. Using the entire patient population, a correlation of increased disease-free survival (DFS) and overall survival (OS) was observed with increased gene expression of IRF2, NCOA1, THAP4, RIPK1, C7orf23 and YTHDC2 ($p<0.05$). Interestingly, decreased DFS and OS of breast cancer patients was related to increased gene expression of LECT2, AGPAT1, CENPN and CETN1 ($p<0.05$). In non-smokers, a correlation was observed between increased DFS and/or OS and increased gene expression of C7orf23, YTHDC2 and IRF2 ($p<0.05$). Decreased DFS and/or OS was associated with increased expression levels of AGPTA1, CENPN, CETN1 and MSX1 ($p<0.05$). In patients with a smoking history, breast carcinomas exhibited a correlation between increased DFS and OS and increased gene expression levels of IRF2, NCOA1, THAP4 and RIPK1 ($p<0.05$). Furthermore, decreased DFS and OS of breast cancer patients was correlated with increased LECT2 gene expression ($p<0.01$). Collectively, results illustrate that although smoking history may not be an independent factor determining breast cancer progression, exposure of a patient to tobacco smoke combined with certain molecular phenotypes of breast carcinoma may alter clinical behavior of this disease. Supported in part by a grant from the Phi Beta Psi Charity Trust (TSK & JLW) and a Research of Women (ROW) grant to JLW from the EVP for Research and Innovation, University of Louisville.

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Novel combination therapy of DNMT inhibitor Guadecitabine and PARP inhibitor Talazoparib for BRCA-deficient and –proficient breast and ovarian cancers

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Breast and ovarian cancer recurrence is associated with increased DNA damage response (DDR) mediated by poly-(ADP)-ribose polymerase 1/2 (PARP1/2), which can be therapeutically targeted by PARP inhibitors (PARPi). PARPi were recently indicated for platinum-responsive, BRCA-mutated high-grade serous ovarian cancer, but most ovarian and breast cancer patients have BRCA-proficient disease. Based on our previous studies supporting a role for DNA methylation in chemoresistant ovarian cancer, mediated by the enzyme DNA methyltransferase 1 (DNMT1), and reports on a functional role for DNMT1 in DNA damage repair, we hypothesized that combining DNMTi and PARPi will impair BRCA-mediated DDR, resulting in cytotoxicity in both breast and ovarian cancer cells. A panel of breast cancer cell lines (MCF7 (BRCA-wt), MDA-MB-231 (BRCA-wt), and SKBR3 (decreased BRCA1 expression)) and ovarian cancer cell lines (A2780 (platinum sensitive, BRCA-wild type (wt)), A2780-cp and HeyC2 (platinum resistant, BRCA-wt) and Kuramochi (platinum resistant, BRCA2 mutant) were examined for cell growth using colony formation assays after treatment with DNMTi guadecitabine (5, 20, or 100nM) and PARPi talazoparib (1 or 10nM), alone or in combination. Combination treatment schemas consisted of: 1) “priming”: Guadecitabine for three days, 24 hour recovery, then talazoparib treatment on Day 5 or 2) “co-administration”: talazoparib administration on day 1 and guadecitabine treatment on days 1-3. In breast cancer cell lines, colony formation was reduced ($P<0.05$) by guadecitabine or talazoparib alone (dose-dependent manner); however, combination drug treatments resulted in greater ($P<0.05$) reduction of colony formation, regardless of BRCA expression. Similarly, in ovarian cancer cell lines, talazoparib alone reduced ($P<0.05$) colony formation (all cell lines, dose-dependent manner), while combination drug treatments again resulted in a greater ($P<0.05$) decrease in percent survival. To focus specifically on the effect of the BRCA status on treatment we utilized two ovarian cells lines derived from the same patient, differing notably in their BRCA2 status: PEO1 (BRCA2 mutant) and PEO4 (BRCA2-wt). Performing Western blot analysis we observed guadecitabine treatment increased ($P<0.05$) PARP levels and enzymatic activity ($P<0.05$), while talazoparib treatment increased ($P<0.05$) DNMT1 recruitment, and decreased PARP activity ($P<0.05$). In addition, we observed, by caspase 3 cleavage, increased ($P<0.05$) apoptosis following combination treatment in either cell line, demonstrating that the combination treatment was not only cytostatic, but also cytotoxic. In summary, talazoparib combined with guadecitabine displayed increased cytotoxicity in both ovarian and breast cancer cell lines harboring either wt- or mutant-BRCA, indicating that this DNMTi-PARPi drug combination impairs BRCA-mediated DDR and may represent an effective treatment regimen for BRCA-related cancers.

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