Tier 1: Basic Science

PI: Cary Moody, PhD, associate professor, Department of Microbiology and Immunology

Project Title: Replication Stress and Survival of HPV Infected Cells

Abstract
Human papillomaviruses (HPV) are associated with multiple human cancers. The HPV oncoprotein E7 drives entry into the cell cycle to provide an environment conducive to productive viral replication in differentiating cells of the stratified epithelium. However, unscheduled cell cycle entry results in replication stress that can lead to genomic instability and cancer development. HPV-infected cells exhibit constitutive activation of the ATR/Chk1 DNA damage response, which is activated by replication stress and is necessary for viral replication. We have found that HPV utilizes the ATR pathway to upregulate RRM2, a key component in nucleotide synthesis that is necessary for viral replication. Cancer cells typically exhibit high levels of replication stress, and ATR-driven accumulation of RRM2 is critical for their survival through supplying dNTPs for DNA repair. RRM2 is also necessary to bypass oncogene-induced senescence (OIS) in response to replication stress. Although our data indicate that HPV utilizes ATR/Chk1/RRM2 activity to facilitate viral replication through increasing dNTP pools, this pathway may also be necessary to combat E7-induced replication stress to promote cell survival. *We hypothesize that HPV infected cells are addicted to the replication stress-protective role of ATR/Chk1/RRM2*. We will determine if HPV utilizes the ATR/Chk1/RRM2 pathway to protect cellular and viral DNA from replication stress, as well as to bypass OIS to facilitate cellular immortalization and viral persistence. Understanding how ATR activity is utilized by HPV will provide insight into mechanisms of viral persistence, as well as genomic instability. These studies may identify therapeutic targets for the treatment of HPV-associated diseases.

Tier 1: Clinical/Translational

PI: Cary Yuchao Jiang, PhD, assistant professor, Department of Biostatistics, UNC Gillings School of Global Public Health

Project Title: Cross-Technology Inference of Tumor Heterogeneity

Abstract
Cancer is a disease driven by rounds of genetic and epigenetic mutations that follow Darwinian evolution. The tumor for a given patient is often a mixture of genotypically and phenotypically distinct cell populations. This contributes to failures of targeted therapies and to drug resistance, rendering the importance of studying intra-tumor heterogeneity. Recently, there have been increasing efforts to sequence longitudinally and/or spatially separated tumor resections from the same patient. Despite much progress, bulk DNA sequencing (DNA-seq) does not allow the characterization of small clones and often times return multiple tumor phylogenies that are equally supported by the data input. Single-cell RNA sequencing (scRNA-seq) circumvents the averaging artifacts associated with traditional bulk population data and offers new opportunities to track cancer clonal evolution at a much finer resolution. This, at the same time, requires new analysis approaches. **(1)** Existing scRNA-seq studies in cancer typically ignore DNA heterogeneity, which is critical since it characterizes cancer subpopulations and fosters tumor evolution. It remains unclear how transcriptomic variation and cellular architecture are modulated by genetic evolution. **(2)** There are no existing methods that profile somatic mutations and reconstruct tumor phylogeny by both bulk DNA-seq and scRNA-seq of the *same* temporally/spatially separated samples from the *same* patient. Building upon our expertise in developing statistical methods and our experience in analyzing cancer genomic and single-cell transcriptomic data, we propose novel frameworks to address the aforementioned problems and some of the key analytical challenges in profiling cancer evolution by both bulk DNA-seq and scRNA-seq.

Tier 1: Clinical/Translational

PI: Matthew R. Redinbo, PhD, Kenan Distinguished Professor, Department of Chemistry
Co-Investigator: Hanna K. Sanoff, MD, MPH, associate professor, Department of Medicine

Project Title: Does the Composition of the Gut Microbiota Affect Clinical Outcomes with Irinotecan?

Abstract
We seek to determine why 30% of patients who receive the anticancer drug irinotecan experience dose-limiting gastrointestinal (GI) toxicity, while the majority tolerate irinotecan with more moderate gut damage. This is an important question because irinotecan is used widely to treat colorectal and pancreas cancers, and gut toxicity leads to dose-reductions that limit clinical efficacy. The Contact PI demonstrated that the GI microbiota cause irinotecan toxicity by producing damaging irinotecan metabolites in the gut lumen. In unpublished mouse data, he has also shown that irinotecan dramatically increases the gut levels of potentially pathogenic Proteobacteria, including opportunistic Enterobacteriaceae like *Salmonella, Klebsiella* and *Shigella*, and Verrucomicrobia, including mucin-degrading *Akkermansia mucinophila* bacterium. The levels of the primary gut bacterial Bacteroides and Firmicutes phyla also change. Thus, we hypothesize that human patients with irinotecan-induced GI toxicity are those with more Proteobacteria and/or Verrocomicrobia in their GI microbiota prior to treatment, or have a greater increase in these taxa during treatment. We will test this hypothesis by enrolling 30 patients at the UNC NC Cancer Hospital, and collecting fecal samples prior to and at four times during irinotecan treatment. We will examine feces with 16S rRNA deep-sequencing to determine the gut microbiota composition at the onset of treatment, and how the composition might change for each patient during treatment. Finally, we will correlate that information with patient-reported and clinically-recorded common terminology criteria for GI toxicities via PROCTCAE and CTCAE, respectively. Taken together, these will be the first human clinical data designed to unravel the role the gut microbiota play in irinotecan outcomes, and have the potential to lead to diagnostic or therapeutic interventions that improve cancer care.

Tier 1: Clinical/Translational

PI: Kyle Wang, MD, resident, Department of Radiation Oncology
Co-Investigator: Lawrence B. Marks, MD, Professor and Chair, Department of Radiation Oncology

Project Title: Prospective Evaluation of Parotid Sparing Whole Brain Radiation and Patient Reported Xerostomia

Abstract
Whole brain radiation (WBRT) is a common treatment for patients with brain metastases. Patients receiving whole brain radiation usually have poor prognoses and maintaining quality of life is important. With the use of traditional opposed-lateral radiation “fields”, both parotid glands are incidentally irradiated. This “collateral damage” is not widely recognized, and the associated adverse effects are underappreciated. Indeed, until recently there have been no studies assessing the incidence/severity of dry mouth in patients receiving WBRT. For this reason, we evaluated patient-reported symptoms in an ongoing prospective observational trial (LCCC 1540: Prospective Evaluation of Patient Reported Xerostomia After Whole Brain Radiation). An interim analysis demonstrated that patients receiving WBRT commonly experienced severe and sometimes persistent dry mouth, with the severity of symptoms related to radiation dose received by the parotids. In this proposed follow-up study, we will investigate whether parotid-sparing WBRT, with field borders defined to explicitly reduce incidental parotid irradiation, can reduce or prevent patient-reported dry mouth. Using a validated patient-reported outcome measurement tool, the Xerostomia Questionnaire, outcomes with parotid-sparing WBRT will be assessed. Further, the results from this approach will be compared to those with conventional techniques (eg, patients treated on LCCC 1540). The results from this study may challenge the long-established paradigm of WBRT planning, arguing for standard delineation and sparing of parotid glands to minimize dry mouth and maximize quality of life in this vulnerable patient population.

Tier 1: Population Science

PI: Jennifer L. Lund, PhD, assistant professor, Department Epidemiology, UNC Gillings School of Global Public Health

Project Title: A multi-database approach to evaluating targeted therapy utilization, sequencing, and adherence in patients diagnosed with metastatic renal cell carcinoma

Abstract
New targeted therapies, including tyrosine kinase inhibitors (TKIs) and mechanistic target of rapamycin (mTOR) inhibitors, have dramatically changed the clinical landscape for metastatic renal cell carcinoma (mRCC). Since 2005, nine new targeted therapies have received Food and Drug Administration approval. This influx of targeted therapies for mRCC presents unique challenges in clinical practice, as: (1) most therapies are orally administered in the home, raising concerns about medication adherence; and (2) patients who initiate first-line targeted therapies often require a second-line agent due to treatment failure, drug resistance, or toxicity. Yet, little is known about the real-world utilization, sequencing, and adherence to targeted therapies for mRCC. We propose to augment the evidence base regarding mRCC targeted therapy delivery by: (1) developing an algorithm to identify mRCC using healthcare claims data and machine learning methods and (2) pooling data to obtain sufficient study samples to conduct descriptive analyses of targeted therapy delivery in routine clinical practice. This study will draw upon four large healthcare databases available to researchers at UNC: (1) the Surveillance, Epidemiology, and End Results (SEER)-Medicare database, (2) a 20% nationwide sample of Medicare data, (3) the MarketScan Commercial Claims and Encounters database, and (4) the Cancer Information and Population Health Resource (CIPHR) data. Preliminary data from this grant will be used to support an NIH application responding to the announcement: "Oral Anticancer Agents: Utilization, Adherence, and Health Care Delivery" that will apply our proposed methods to other tumor types and ultimately inform interventions to improve patient access and outcomes.

Tier 1: Population Science

PI: Katherine E Reeder-Hayes, MD, MBA, MSCR, assistant professor, Department of Medicine

Project Title: Health System Impacts on Care and Survival of Solid Tumor Brain Metastases

Abstract
Brain metastasis is a common complication of solid tumor cancers which confers high burdens of morbidity, treatment toxicity, and cost, as well as a poor prognosis. While evidence from randomized clinical trials provides guidance in selecting appropriate patients for treatment with focal or whole brain radiotherapy or surgery, little is known about the impact of health system factors on patterns of care, or about how the choice of first line therapy impacts downstream cancer care needs and use of salvage therapies. The Lineberger Comprehensive Cancer Center Brain Metastasis Clinic provides multi-disciplinary care in a unique delivery model, but care delivery to patients around the state could be enhanced by a better understanding of the burden and patterns of care of brain metastases in North Carolina. We propose to use the resources of the Cancer Information and Public Health Resource, which links North Carolina cancer registry data to insurance claims from multiple payers, to investigate the current patterns of care for patients with brain metastases in North Carolina as they relate to patient-level, provider-level and geographic factors, and how these patterns influence healthcare utilization over the patient's lifetime. We will also provide detailed prognostic estimates specific to each tumor type and histology and examine trends in post-treatment survival over time.

Tier 2: Basic Science

PI: Lee M. Graves, PhD, professor, Department of Pharmacology
Co-Investigator: Matthew Lockett, PhD, assistant professor, Department of Chemistry
Project Title: Characterization of a Novel Class of Imipridone Molecules for Cancer Research

Abstract
Onc201 (Oncoceutics) is a small molecule currently in clinical trials as a first-in-class treatment for glioblastoma and other cancers. While believed to induce cell death through inhibition of ERK/Akt signaling and induction of the pro-apoptotic TRAIL ligand, this imipridone class of molecules is poorly characterized. Other research suggests that Onc201 is acting as a dopamine receptor antagonist. We have a series of novel Onc201 analogs (the TR-compounds) and have shown superior potency of these over Onc201 in multiple cancer models. Our goals of this proposal are twofold; to identify the cellular targets for the TR-compounds and to evaluate their efficacy in 3D co-culture models of triple negative breast cancer (TNBC). Proteomics tools will be used to determine if there are direct kinase or non-kinase targets for the TR-compounds. We have created a TR compound affinity resin for the purpose of identifying TR binding proteins by mass spectrometry. In collaboration with Bryan Roth, large-scale GPCR screens will be performed to determine if these compounds are dopamine receptor or other GPCR antagonists. In our second aim, we will examine the effects of the TR-compounds in unique 3D culture platforms developed by the Lockett lab. This platform models increasing stages of breast cancer progression—from ductal carcinoma in situ to invasive ductal carcinomas and is able to support co-cultures in experimentally defined oxygen and nutrient gradients. We will separate and evaluate populations of live cells along these gradients with phenotypic assays, transcript profiles, and multiplexed immunoassays. Simultaneous evaluation of cancer and CAF responses will determine if the microenvironment influences cellular responses, drug selectivity, and the mechanism of action. Ultimately we aim to progress a lead compound into UNC Mouse Phase I trials to establish efficacy in established mouse models of breast cancers.

Tier 2: Basic Science

PI: Laurianne Van Landeghem, PhD, assistant professor, Neurogastroenterology, Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University

Co-Investigator: Scott Magness, PhD, associate professor, Departments of Medicine, Cell Biology & Physiology; Joint Department of Biomedical Engineering, North Carolina & North Carolina State University

Project Title: Remodeling of the Enteric Glial Network in Colon Cancer

Abstract
Colon cancer initiation, invasion, metastasis and relapse is driven by a subset of cancer cells, termed cancer stem cells (CSC), that possess increased tumor-initiating abilities, chemoresistance, invasiveness and metastatic potential. Compelling evidence have demonstrated that CSC are controlled by the so-called tumor microenvironment composed of resident and recruited cells that have been altered/remodeled by the tumor to sustain its growth and dissemination. Among the cell types of the tumor microenvironment are enteric glial cells (EGC), which form a dense network immediately adjacent to tumor epithelial cells. However whether EGC of the tumor microenvironment participate to colon carcinogenesis has never been studied. Preliminary work using cell lines suggests that EGC are activated by tumor-derived paracrine factors to become pro-tumorigenic *via* increased glial PGE2. Furthermore we have evidence that once activated by the tumor, EGC induce CSC expansion and tumor-initiating abilities *via* a EP4/EGFR-dependent pathway. Also one recent descriptive study has reported alterations in the glial network in human colon adenomas. Thus this proposal will test that EGC remodeling is an early event during cancer progression that induces CSC expansion in colon tumors. Using exclusively human primary cells, we will characterize the remodeling of the EGC network over the course of cancer progression by iDISCO volume imaging, single-cell RNAseq and mass spectrometry, and assess its impact on CSC expansion and

tumor-initiating abilities using *in vitro* paracrine co-culture 3D models and *in vivo* (co-)engraftment systems. Proposed studies are likely to identify molecular targets to block EGC remodeling and/or EGC/CSC crosstalk, hence colon carcinogenesis.

Tier 2: Basic Science

PI: David A. Zaharoff, associate professor, Department of Biomedical Engineering

Co-Investigator: Benjamin G. Vincent, MD, assistant professor, Division of Hematology/Oncology
Project Title: Characterization of the neoantigen-specific T cell response following intravesical chitosan/IL-12 immunotherapy

Abstract
Bladder cancer, the 6th most common non-cutaneous cancer diagnosis in the U.S., is a highly recurrent disease which often requires long-term maintenance therapy and continuous surveillance. Novel therapies capable of inducing durable anti-tumor immunity are needed to limit recurrences and metastatic progression of bladder cancers. Interleukin-12 (IL-12) is a potent, anti-tumor cytokine. Recently, intravesical administrations of IL-12 co-formulated with the mucoadherent biopolymer, chitosan (CS/IL-12) were found to: 1) eliminate established orthotopic bladder tumors; 2) generate tumor-specific immunity; and 3) induce abscopal responses in preclinical models. Nevertheless, a better understanding of the antigen specificity of the T-cell response and the kinetics of T cell memory is needed prior to clinical translation of intravesical chitosan/IL-12 immunotherapy. This project leverages recent immunogenomic analyses of bladder tumors and next generation sequencing of T-cell receptor repertoires to study the induction and amplification of neoantigen specific T-cells in response to intravesical CS/IL-12. The proposed research will answer two questions: 1) does CS/IL-12 immunotherapy amplify pre-existing T-cell clones or expand T-cell diversity through the recruitment of new clonotypes (or both)?; and 2) when, i.e. after how many treatments, is tumor-specific immune memory established? Results of this project are expected to demonstrate that CS/IL-12 is a universal immunotherapy capable of generating personalized, neoantigen-specific immunity by inducing an *in situ* tumor vaccination. Because intravesical CS/IL-12, unlike current treatments, can induce bladder tumor-specific immunity, there is considerable interest in translating this novel immunotherapy at the UNC LCCC. Data gathered will inform clinical trial design and directly support a first-in-human clinical study.

Tier 2: Clinical/Translational

PI: Jenny P-Y Ting, PhD, William R. Kenan, Jr. Distinguished Professor, Department Microbiology and Immunology

Co-Investigator: Kristy Ainslie, PhD, associate professor, UNC Eshelman School of Pharmacy

Project Title: A Novel Microparticle Platform to Activate Innate Immunity as an Immunotherapeutic for Triple Negative Breast Cancer

Abstract
Triple negative breast cancer (TNBC – estrogen receptor, progesterone receptor, and HER2 negative) patients have poorer 5-year survival compared to other breast cancers, due in part to the lack of viable therapies - underscoring the need for novel therapies, such as immunotherapy. Unfortunately, conventional immunotherapy has only modest effects in controlling TNBC. Furthermore, cancer immunotherapy targets only the acquired immune system, while neglecting the innate immune system. Pathogen Associated Molecular Patterns (PAMPs) are molecules that activate innate immunity through binding to their cognate receptors. However, a major hurdle to the implementation of PAMPs as cancer immunotherapies is the intracellular localization of many receptors. We have overcome this hurdle using microparticles (MPs) composed of acetalated dextran (Ac-DEX) that is safe, stable, easy to sterilize and manufacture. Using Ac-DEX MPs we can deliver PAMPs to intracellular receptors in

innate immune cells. Preliminary studies show that this platform enhances anti-tumor activity of PAMPs in TNBC through remodeling the immune-microenvironment of the tumor, leading to cytotoxic T cell responses. This proposal will expand on these findings by comparing multiple PAMPs with known anti-tumor activity, delivered via Ac-DEX MPs. Candidate Ac-DEX PAMP MPs that show significant anti-tumor activity will be further evaluated in combination with conventional therapeutic approaches in three TNBC models which differ in their genetic background, and in their responsiveness to chemotherapy, radiation, and checkpoint blockade. Furthermore, we will initiate mechanistic studies to reveal immunological mechanisms of anti-tumor activity. These studies will serve to identify therapeutic candidates for clinical translation and additional NIH funding.

Tier 2: Clinical/Translational

PI: Sarah J. Nyante, MSPH, PhD, assistant professor, Department of Radiology

Co-Investigator: Jay Crawford, MHA, informatics manager, Louise Henderson, MSPH, PhD, associate professor, and Cherie Kuzmiak, MD, associate professor, Department of Radiology; and Di Wu, PhD, assistant professor, Department of Biostatistics

Project Title: Quantitative Imaging Data in a Community-Based Mammography Registry: A Feasibility Study

Abstract

Mammographic density is linked to both breast cancer risk and the ability to detect breast tumors. As such, accurate mammographic density assessment is critical for developing breast cancer screening and risk management strategies. In a majority of imaging practices, mammographic density is assessed visually by a radiologist using a four-level scale. However, such visual assessments are subjective and have poor reproducibility compared with measurements from automated software. Moreover, automated software calculates mammographic density as a quantitative, continuous measure (percent density), which allows for greater precision. Despite these advantages, quantitative density is rarely included in epidemiologic studies, as the number of well-characterized populations that have breast images from which quantitative density can be measured is limited. In this study, we propose to establish an image bank within the Carolina Mammography Registry (CMR) to enable the integration of percent mammographic density into studies of breast cancer screening, risk, and survival among women in North Carolina. The CMR is a prospective, community-based breast imaging registry that collects information on demographics, screening history, risk factors, imaging exam characteristics, and benign and malignant cancer diagnoses. We will use a case-cohort design, in which the first case group will consist of women recalled for additional imaging after a screening mammogram. In this way, we will evaluate the association between quantitative density and screening recall, while laying the foundation for future studies of how density and other quantitative breast composition measures may affect breast tumor detection and tumor characteristics.