Tier 2-Basic Science

PI: Leslie M. Hicks, PhD, Associate Professor, Department of Chemistry

Co-Investigator: Janelle Arthur

Project Title: Colibactin induction in genotoxic pks+ E. coli and its role in DNA-damage and colorectal cancer

Abstract

Colibactin is a bacterial genotoxin that augments colorectal cancer (CRC) through enhanced DNA damage. Physical contact of pks+ E. coli with host epithelial cells is required, which results in alkylation of host DNA and a unique mutational fingerprint. The pks island is a hybrid NRPS-PKS cluster encoding nineteen genes whose regulation is poorly understood. Although much has been done to elucidate the molecular structure and biosynthesis, colibactin's mechanism of delivery and microbiological function remain unknown even fourteen years after its discovery. We have evolved resistance in two distinct strains of pks+ E. coli with an antibiotic, resulting in up to 36-fold increased expression of PKS proteins. Further, we have shown that this increase in biosynthetic protein expression results in elevated DNA damage in vitro. In this work, we will implement in vitro and in vivo techniques such as label-free quantitative proteomics and gnotobiotic mouse models to exploit and understand this intriguing phenomenon and further examine colibactin's role in CRC development.

Tier 2 – Basic Science

PI: Yanguang Cao, Assistant Professor, Pharmacotherapy and Experimental Therapeutics

Co-Investigators: Gianpietro Dotti

Project Title: Measuring CAR T cell–tumor cell interactions in vivo to overcome resistance to CAR-T therapy in solid tumors.

Abstract

Despite the success in B–cell hematologic tumors, the efficacy of CAR-T therapy against solid tumors is still unsatisfactory. Two elements of CAR-T success in hematologic tumors – high CAR-T cell expansion and potent tumor cytolytic effect – are both initiated and defined by a fundamental process: the intercellular interactions between CAR-T and tumor cells. Unfortunately, the dynamics of their interactions in solid tumors and the desirable features of CAR-T cells that can survive and effectively interact with tumor cells remain largely undefined, mostly because an approach for measuring CAR-T and tumor cell interactions directly in vivo is still lacking. Hence, the current project aims to develop an enzymatic intercellular labeling approach for measuring and modeling CAR-T and tumor cell interactions in vivo. Specifically, we will apply an intercellular proximity labeling enzyme (an engineered Staphylococcus aureus transpeptidase Sortase A, mgSrtA) in tumor cells to mark the CAR-T cells that have undergone interactions with tumor cells. These in vivo labeled–CAR-T cells are detectable and sortable by flow cytometry for downstream profiling. As a tumor model we will leverage on our recently developed model of pancreatic ductal adenocarcinoma (PDAC) in immunocompetent mice and the B7H3-specific CAR-T cells. The intercellular labeling approach will characterize the cellular and molecular features of B7-H3.CAR-T cells that can effectively interact with tumor cells in the TME. The completion

of this project will identify the subset of CAR-T cells that can survive, function, and overcome resistance in solid tumors.

Tier 2 – Basic Science

PI: Bernard E Weissman PhD, Professor, Pathology and Laboratory Medicine

Co-Investigator: Robert C Smart

Project Title: Role of activated NRF2 signaling in the development of cutaneous squamous cell carcinoma

Abstract

Despite advances in early detection and treatment strategies, the five-year survival rate for most squamous cell carcinoma (SCC) patients remains dismal (<10%). To identify new therapeutic targets, next-generation sequencing of SCCs revealed a significant number of activating mutations in the KEAP1-NRF2 pathway. NRF2's activity, regulated by the ubiquitin ligase KEAP1, modulates a battery of cytoprotective genes against stress. Importantly, patients with cancers possessing NRF2 mutations display poorer survival and greater resistance to standard treatments. Thus, a need exists to elucidate the mechanisms by which mutant-NRF2 promotes cancer growth and survival. To discover mechanisms by which mutant-NRF2 drives SCC development, we generated a Nrf2E79Q/+ GEMM possessing one of the most common NRF2 activating mutations found in human SCC within the endogenous mouse Nrf2 locus. Using this model, we will determine the impact of mutant-NRF2 signaling on tumor development, progression and metastasis in the classic cutaneous SCC model. We hypothesize that mutant-NRF2 signaling will promote the progression of cutaneous SCC through novel downstream pathways. We will combine the expertise of 2 investigators, Dr. Robert Smart, a NC State Professor and Dr. Bernard Weissman, a UNC Professor, to perform the following aims: AIM#1: Determine the effects of mutant Nrf2E79Q expression on the development of squamous papillomas and their progression to SCCs and AIM#2: Identify transcription targets unique to mutant NRF2 in epidermis, papillomas and SCCs. Our research will help develop targeted drug therapies for human SCCs with frequent NRF2 mutations such as cutaneous, bladder, head and neck and esophagus.

Tier 2 – Basic Science

PI: Kirsten Bryant, PhD, Research Assistant Professor, Pharmacology

Co-Investigator: Yuliya Pylayeva-Gupta

Project Title: Identification of potent and selective modulators of pancreatic cancer cell autophagy as potential therapeutics

Abstract

Mutational activation of the KRAS oncogene is found in ~95% of pancreatic ductal adenocarcinoma (PDAC) and continued KRAS function is essential for maintenance of PDAC tumorigenic growth. The crucial role of KRAS as a cancer driver is mediated, in part, by its deregulation of metabolic processes that support the increased energy needs of cancer cells. We recently determined that genetic suppression of KRAS or pharmacologic inhibition of its key effector signaling pathway, the RAF-MEK-ERK mitogen-activated protein kinase (MAPK) cascade, blocked two critical metabolic processes (glycolysis

and mitochondrial function), yet unexpectedly elevated a third, autophagy. Autophagy is a metabolic process of self-eating and recycling of defective organelles for nutrient production. Speculating that ERK MAPK inhibition rendered PDAC addicted to autophagy, we then showed that concurrent treatment with inhibitors of ERK MAPK and the autophagy inhibitor hydroxychloroquine (HCQ) caused synergistic apoptotic death of PDAC in vitro and in vivo. Our findings provided the rationale for our initiation of Phase I/II clinical trials combining inhibitors of MEK/ERK and HCQ as a new therapeutic strategy for PDAC. HCQ is the only autophagy inhibitor in clinical use yet is limited in both potency and selectivity. To develop improved therapeutic strategies for targeting autophagy, we will establish and apply a fluorescence-based high-throughput PDAC-based autophagy screen for evaluation of the Published Kinase Inhibitor Sets (PKIS) of ~1,000 ATP-competitive protein kinase inhibitors. Our screen will identify novel protein kinases that inhibit or promote autophagy and serve as new therapeutic strategies for targeting autophagy for PDAC treatment.

Tier 2 – Clinical

PI: Jason D Merker, MD, PhD, Associate Professor, Departments of Pathology and Laboratory Medicine & Genetics

Co-Investigators: Michael Ramsey, Co-PI; Paul Armistead; Matthew Foster; Nathan Montgomery; Joel Parker; Sushant Patil

Project Title: Development and application of a novel digital array PCR assay for evaluation of minimal residual disease in acute myeloid leukemia

Abstract

Minimal residual disease (MRD) refers to the presence of cancer cells that remain after therapy and are not otherwise detectable by standard clinical assays or radiographic imaging. Detection of MRD before it becomes clinically detectable provides an opportunity to intervene and optimize treatment of these patients, with the possibility of curing more patients. In some liquid tumors, MRD assessment is part of routine clinical practice. However, current MRD assays applicable to most cancers have significant limitations, making it challenging to 1) evaluate clinical validity and utility of MRD assays in clinical trials; and 2) subsequently incorporate useful assays into clinical practice. We will adapt a novel, highly multiplexed technology, digital array PCR (daPCR), for evaluation of MRD. This UNC-developed technology provides a high throughput, multiplexed platform with unprecedented sensitivity, high ease of use, low cost and rapid turnaround time. These technological advances provide a real-world solution to enable frequent, deep monitoring of therapeutic response in patients with cancer. For an initial application of this technology, we will develop and pilot an assay for MRD assessment in the liquid tumor acute myeloid leukemia (AML), since MRD assessment has demonstrated clinical validity in this malignancy. To apply this technology, we have brought together a team with deep expertise in technology development, molecular diagnostics, AML biology and therapy, and bioinformatics. Following successful application in AML, this technology can be readily applied to other cancer types.

Tier 1 – Population Science

PI: Til Strumer, MD, PhD, MPH, Distinguished Professor and Chair, Epidemiology

Co-Investigator: Louise Henderson, Jesper Hallas

Project Title: Statins, metformin and evidence for breast cancer chemoprevention: the role of differential use of screening mammography.

Abstract

Cancer is poised to become the leading cause of death in the 21st century and there is a pressing need to identify new cancer prevention strategies. One of the most promising approaches is to repurpose widely-used and well-tolerated medications that have shown anti-cancer activity in preclinical studies (cancer chemoprevention). Individuals with certain chronic diseases may be at higher cancer risk, and chemoprevention may therefore constitute a precision prevention approach for high-risk groups. Due to their size, generalizability, and low costs, nonexperimental data sources will be critical for this discovery process. However, real-world patterns of healthcare access, quality, and patient and provider behavior shape both medication use and receipt of cancer screening. In the case of screen-detectable cancers like breast cancer, screening can advance disease detection and lead to overdiagnosis. Failure to properly account for differential screening in cancer chemoprevention studies may result in incorrect conclusions regarding medication safety or efficacy, and few studies have attempted to describe or address this potential source of bias. The proposed study will aim to 1) describe differences in screening mammography receipt among users of two proposed breast cancer chemopreventive drugs, statins and metformin, and 2) employ several analytic methods and a novel sensitivity analysis to account for differential screening. Preliminary data from this grant will be used to support an application responding to the NIH funding announcement Development of Innovative Informatics Methods and Algorithms for Cancer Research and Management that will allow us to replicate our findings in more heterogenous populations and care settings.

Tier 1 – **Population Science**

PI: **Angela M. Stover PhD, Assistant Professor, Health Policy and Management**

Co-Investigators: Ethan Basch, Anna Beeber, Arlene Chung, Darren DeWalt, Angie Smith

Project Title: Improving patient outcomes by partnering with oncology clinics to implement symptom monitoring with EHR-embedded patient reported outcome measures

Abstract

Distress and pain are higher among African American, older, and rural cancer patients, yet these groups are less likely to be screened for burdensome symptoms during their care. Self-report questionnaires called electronic patient-reported outcomes (ePROs) assess how individuals feel and function, and can be integrated in electronic health record systems to universally screen for symptoms, which may reduce the screening disparity. Health systems and patients are interested in using ePROs at clinic visits, but implementation is challenging because of necessary workflow changes and training clinic teams. This Lineberger award will support our team in examining optimal methods to support North Carolina Cancer Hospital (NCCH) clinics in implementing ePROs to improve symptom screening rates and clinical outcomes. In Aim 1, we will elicit key stakeholder perceptions about the ePRO implementation process, barriers, enablers, and implementation support strategies from oncology clinic teams and the implementation team at UNC Health Care. In Aim 2, we will examine patient- and clinic-level correlates of ePRO completion rates (reach/service penetration of ePROs) in NCCH clinics. This study is significant because (1) universal screening with ePROs may reduce disparities in screening for undertreated

symptoms in at-risk cancer populations, (2) this study will provide the foundation on optimal support strategies for clinics implementing ePROs, and (3) results of this award will be used to support R01 applications to understand how, why, and in what circumstances ePRO implementation is successful. Aim 2 will also establish a process for extracting EPIC ePRO data from the Carolina Data Warehouse for Health.

Tier 1 – Clinical

PI: Marc A Bjurlin, DO, MSc, Associate Professor, Urology

Co-Investigator: William Kim MD, Rebecca Frye PhD

Project Title: Evaluating the carcinogenic effects of electronic cigarettes on the urothelium

Abstract

Electronic cigarette (e-cigarette) use is a public health crisis in the United States, with an exponential rise in its use over the last 3 years. E-cigarettes are considered an alternative to conventional cigarettes and their appealing appearance, taste and use characteristics make them popular among many age groups. Similar to combustible tobacco smokers, e-cigarette users are exposed to a variety of toxic and carcinogenic compounds during the vaping process. Specifically, carcinogenic compounds as well as their metabolized byproducts have been observed in the urine of e-cigarette users that have a strong link to the development of bladder cancer. Cigarette smoking produces specific bladder carcinogens which get converted into reactive intermediates that interact with DNA and have potential mutagenic consequences. Urinary epigenetic changes, such as DNA hypermethylation of innate immunity genes and telomerase reverse transcriptase (TERT) promoter mutations, are initial events in the oncogenesis of bladder cancer and are detectable in urinary DNA. Since similar carcinogenic compounds of smokers have been observed in the urine of e-cigarette users, we hypothesize that comparable DNA methylation changes and TERT promoter mutations are occurring in e-cigarette users which may place them at risk for the development of bladder cancer. To address this health concern, we propose to assess the DNA methylation changes of bladder cancer related genes and prevalence of TERT promoter mutations in the urine of e-cigarette users compared to non-users, cigarette smokers, and bladder cancer patients. These studies will assist the understanding of potential bladder cancer risk of e-cigarette use.

Tier 1 – Basic Science

PI: Brian Kuhlman PhD, Professor, Biochemistry and Biophysics

Project Title: Engineering dual targeting of CD19 and CD20 to mitigate tumor antigen escape during CAR T-cell therapy

Abstract

Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of B cell non-Hodgkin lymphoma and acute lymphoblastic lymphoma. However, a subset of patients experience relapse because of loss of the target antigen (CD19) on their cancer cells. This discovery has prompted researchers to explore approaches for engineering T cells that can target multiple cell surface receptors so that cell killing is maintained even if one of the receptors is lost. One approach actively being studied is the use of CARs that contain multiple antigen binding domains fused together via linkers. However, in

preliminary studies the efficacies of these tandem CARs have been lower than what is observed with monospecific CARs. The decrease in activity may be due to sub-optimal placement relative to the cell surface or could stem from the fact that the antibody fragments used in CARs do not properly fold when placed in tandem. Here, we propose an alternative solution that avoids these limitations. Using molecular modeling and yeast cell surface display we will engineer a single antibody fragment (scFv) that can bind both B-cell receptors CD19 and CD20. Previous studies have demonstrated the feasibility of engineering antibodies that recognize two antigens (so called two-in-one antibodies), but this strategy has yet to be tested in the context of CAR T-cell therapy. We will validate our engineered scFvs with stability and binding studies, and promising candidates will be used in future animal and clinical studies in collaboration with Dr. Dotti's group at UNC.

Tier 1-Basic Science

PI: Andrea A Hayes-Jordan MD, Distinguished Professor, Department of Surgery

Project Title: Identifying Genetic Susceptibility in Human Desmoplastic Small Round Cell Tumors.

Abstract

Desmoplastic small round cell tumor (DSRCT) is a rare malignant tumor that predominantly affects pediatric, adolescents and young adult males. Long term survival is dismal ranging from 15-30%. Approximately 90% of patients are male and most present with diffuse (dozens to hundreds) of abdominal tumors, in addition to some with liver lymph node and lung metastasis. The organ or tissue of origin is unknown. We hypothesize the organ of origin of DSRCT is androgen sensitive and dependent on the microenvironment.

For the purpose of this 1 year pilot grant, our specific aim is to identify the genetic susceptibility of DSRCT cancer stem cells (CSC), and therefore potential targets of therapy in the androgen sensitive microenvironment. We further hypothesize there are cancer stem cells resistant to chemo/radio-therapy which could be a progenitor for the initiation and recurrence of DSRCT. In our preliminary data we successfully performed single cell sequencing and complete cell type assignment based on the marker genes showed the expected groups of DSRCT tumor cells, stromal cells, immune cells, endothelial cells. We found a distinct cluster of potential cancer stem cells, which specifically express both DSRCT markers and cancer stem cell markers. Androgen receptor is also highly expressed in these cells. The pluripotency and tumor initiating potential of these cells will be characterized in vitro and in vivo. The androgen agonists and antagonists will be administrated in vitro and in vivo, to investigate the role of androgen signaling on the growth and invasion of the CSC in DSRCT.

Tier 1 – Basic Science

PI: Allen Cole Burks, MD, Assistant Professor, Internal Medicine

Co-Investigator: Yueh Lee, Gianmarco Pinton

Project Title: Development of a Miniaturized Multidirectional Ultrasound Ablation Array (MiDUSa) for Endobronchoscopic Lung Nodule Ablation

Abstract

Minimally invasive treatment of early stage lung cancer is of increasing interest in the era of Lung Cancer screening and increasing age and comorbidities of the population. Currently available endoscopic ablative techniques, such as radiofrequency, microwave, and cryotherapy have significant drawbacks in practical application either due to design elements or patient safety related concerns. In the lung, ultrasound ablation is attractive due to ultrasound reflective nature of the aerated lung, and the ability to shape and focus the acoustic energy with multiple transducer arrays may provide additional safety elements not capable in other modalities. We propose to simulate energy deposition profiles of candidate multi-element ultrasound arrays and develop a miniaturized multidirectional ultrasound transducer that can be deployed in modern bronchoscopic applications for targeting of peripheral pulmonary lesions. We will then model ablation fields utilizing clinical CT scans of patients that are high cancer risk with peripheral nodules being considered for peripheral bronchoscopic biopsy. The expected outcome is a 2.0-2.6mm diameter multidirectional ultrasound ablation array capable of creating a shapeable and predictable thermal coagulative necrosis profile with a depth of at least 2cm.