Grant 1 R15 CA249796-01A1

PI Name(s) ANANIEVA-STOYANOVA, ELITSA ANTONOVA

Title Elucidating the role of the Branched Chain Aminotransferases (BCATc and BCATm) as novel metabolic checkpoints of anti-lymphoma T cell immunity

Institution DES MOINES UNIV OSTEOPATHIC MEDICAL CTR

Abstract New immunotherapies targeting lymphomas delivered promising results during recent clinical trials. However, these therapies were only effective in a small subset of patients with short periods of remission. The results from these studies suggested the existence of immunosuppression in the tumor microenvironment. Indeed, the lymphoma microenvironment is a very dynamic network between lymphoma cells and non-malignant components that may promote tumor growth and consequently drug resistance. Progress in T cell metabolism has demonstrated that T cells experience a metabolic disadvantage in the tumor microenvironment, which often manifests in T cell exhaustion that jeopardizes their potential to destroy cancer cells. This reveals a critical need to explore new (metabolic) approaches to improve T cell performance. Our research team proposes to target the metabolism of the branched chain amino acids (BCAAs) as a novel metabolic checkpoint of T cell activation in the lymphoma microenvironment. Our rationale stems from the findings that the BCAA, leucine, is indispensable for T cells activation, while BCAA metabolism, initiated by the cytosolic (BCATc) and mitochondrial (BCATm) branched-chain aminotransferases, is a means to direct leucine toward degradation. The objective in this application is to determine whether a loss of expression of BCATc and BCATm is beneficial for the durability and functional integrity of T cells during lymphoma eradication in unique pre-clinical mouse models created in our laboratory. The long-term goal of this application is to provide new means to improve the T cell-mediated immune response and to address the challenges with T cell-driven anti-lymphoma immunotherapy. The central hypothesis is that BCATc, supported by BCATm, serves to provide checkpoint control on T cell function by being a part of a negative feedback loop regulation of T cell activation. Deletion of the BCAT genes from T cells, individually or in combination, may provide a metabolic advantage of T cells allowing them to remain activated and to successfully combat lymphoma growth. To test the central hypothesis, we identified three specific aims: (1) Investigate how the expression of BCATc and BCATm changes upon T cell subset differentiation and whether the BCAT proteins are essential for T cell lineage commitment and function, (2) Determine whether a blockage in the transamination of BCAAs enhances the T cell response to lymphoma tumors, and (3) Investigate whether a loss of expression of BCATc in mouse T cells can overcome the lymphoma resistance to anti-CTLA4 therapy. Completion of this project will not only provide the opportunity to improve the current treatment options for lymphoma patients but will also engage students in pre-clinical cancer studies. The students will highly benefit from acquiring hands-on research experience in cancer, which can be translated into enhanced research skills, scientific reasoning, and better understanding of treatment approaches.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant 1 R15 CA277814-01A1

PI Name(s) BILLINGSLEY, KELVIN L

Title Hyperpolarized 13C Metabolic Imaging of Tumorigenesis in the Liver

Institution LOYOLA UNIVERSITY OF CHICAGO

Abstract Aberrant glycolysis and mitochondrial function are features of most liver diseases including hepatocellular carcinoma (HCC). Despite these metabolic signatures, the absence of methods to noninvasively assess metabolic fluxes in vivo limits the accurate characterization of liver diseases and in turn impedes the development of new therapies. In the proposed study, we will employ novel hyperpolarized (HP) 13C probes to image glycolysis, a pathway that plays a critical role in HCC onset and progression. Importantly, our cross-disciplinary research team has made significant advancements in the design and application of HP 13C-glycerate probes. We have demonstrated that HP [1-13C]glycerate is a non-toxic substrate with a long T1 relaxation time (60 sec), and this HP probe is sensitive to alterations in liver metabolism in vivo, offering inroads for clinical translation. In addition, our recent studies in HCC (diethylnitrosamine [DEN]-induced rat model) demonstrated that HP [1- 13C]glycerate can successfully distinguish HCC from healthy liver based upon the unique metabolic fluxes detected in the cancerous tissue. Given these advancements, we now propose that the HP 13C-glycerate scaffold can be systematically optimized to yield 2nd generation HP probes, which provide highly sensitive analyses of enzymatic reactions in the liver and diagnostic assessments of abnormal fluxes in HCC. The overarching goal of the proposed project is to use the HP 13C-glycerate technology to establish in vivo imaging biomarkers for assessing altered metabolism during HCC development. To this end, in Aim 1, we will synthesize a focused library of 2nd generation 13C-glycerate probes that are specifically designed to increase the metabolic information obtained from HP experiments. In Aim 2, we will analyze the physicochemical properties of these probes in order to determine top agents to advance towards in vivo HP studies. In Aim 3, we will initially establish imaging biomarkers for HP 13C-glycerates in the DEN rat model and identify specific probes that provide clear metrics for distinguishing HCC. These 13C-glycerate probes will then be used to evaluate a stepwise progression from normal liver to HCC in the DEN model. Four pathological states will be examined: baseline, chronic inflammation, fibrosis, and HCC. In vivo metrics for glycolysis will be compared among the states, and these results will be validated with tissue analyses. Overall, the proposed studies offer an innovative strategy for tackling a challenge of clinical significance. State-of-the-art HP probes will be used to assess altered glycolysis in hepatocarcinogenesis. This technology will in turn provide specific in vivo biomarkers that represent the metabolic pathways of interest in HCC, providing a noninvasive method for assessing disease progression in at- risk patients.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director AMIN, ANOWARUL

Grant 1 R15 CA242349-01A1

PI Name(s) BROOKS, CORY

Title Role of Antigen Glycosylation in Mucin Binding by Monoclonal Antibodies

Institution CALIFORNIA STATE UNIVERSITY FRESNO

Abstract Immunotherapy has become one of the central pillars of cancer treatment. The deployment of antibody-based therapies has transformed the lives of thousands of patients. A molecular understanding of how antibodies interact with their targets is invaluable for the development of new successful antibody drugs and immunotherapy products. One of the most important targets for the discovery of new cancer immunotherapies is the mucin family of glycoproteins. Mucin proteins are frequently overexpressed and display altered abnormal glycosylation in several types of adenocarcimona. However, despite the importance of mucin proteins as immunotherapy targets, little is known regarding how antibodies bind these proteins. To address this gap in our knowledge, we propose using a panel of antibodies that bind the mucins MUC1 and MUC16 as models to understand molecular recognition of these important therapeutic targets. Preliminary studies in our lab have demonstrated that glycosylation of MUC1 influences the conformational dynamics of epitopes which in turn influence antibody binding. We will expand our understanding of this phenomenon using a combination of structural biology, computational modeling and binding studies on a panel of MUC1 specific antibodies. Specifically, we aim to determine the role of cancer associated mucin glycosylation in antibody recognition of MUC1 (AIM 1). Previously published results suggest that MUC16 antibodies bind non-linear epitopes localized within the tandem-repeat region of the protein. This region is heavily glycosylated, and the role of MUC16 glycosylation on antibody binding is unknown. Preliminary studies in our lab have determined that a humanized MUC16 antibody binds to an epitope with a SEA domain. Using several therapeutic antibody candidates as models, we propose to employ structural and binding studies to characterize the nature of non-linear MUC16 epitopes recognized by therapeutic antibodies (AIM 2).

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant 1 R15 CA256668-01A1

PI Name(s) CHI, YUJIE

Title Development of the next-generation GPU-based Monte Carlo simulation platform for radiation-induced DNA damage calculations

Institution UNIVERSITY OF TEXAS ARLINGTON

Abstract Ionizing radiation (IR) is a critical component of modern medicine. When IR penetrates through the organism, it could depart its energy to the medium mainly through ionization and excitation. The energy departure of IR is medium composition dependent, and hence it is used to ‘see’ the inner structure of the human beings, enabling the application of IR in the medical imaging of mammography, chest x-rays, computational tomography, positron emission tomography, etc. IR can also damage the structure and/or affect the function of the organism and hence it is applied to treat cancer in the form of radiosurgery and radiotherapy. Meanwhile, IR is found to be genotoxic and carcinogenic, calling the non-ending effort to understand the fundamental effects. Advanced cellular radiobiological study exhibited that the damage of deoxyribonucleic acid (DNA) plays a pivotal role towards the determination of the final biological or even clinical outcome after exposure to IR. It is hypothesized that when IR interacts with DNA, it could damage DNA in picoseconds by the primary and secondary IR particles and in microseconds by subsequently generated radiation radicals. It is then essential to understand how IR produces this initial damage under various radiation conditions. Microscopic Monte Carlo (MC) simulation such as Geant4-DNA, capable of computing this damaging process, has been playing an important role in the quantitative hypothesis-test. However, there are several issues in the state-of-the-art MC tools, making it hard to meet the increasing demanding for advanced applications. These include the low efficiency in dealing with the ‘many-body’ problem, the relatively large uncertainty in the final computing results, the lack of support for the entire cell cycle and the limited-access/user-unfriendly designs, etc. In this project, we propose to solve the above issues by developing a next-generation MC simulation tool for IR induced DNA damage computation through the novel implementations of graphical processing units (GPUs) parallel computing, the molecular dynamics/first principles based computation, the new DNA model development based on the extrusion model and polymer physics, and the open-source release with user-friendly interface. Upon success, the developed system is expected to serve as a next-generation simulation platform for the calculation of the initial DNA damage caused by IR, which can become a profound first-step towards a successful accomplishment of the “bottom-up” multi-scale modeling for the entire radiobiological process, making a significant impact in radiation medicine.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director MILLER, DAVID J

Grant 1 R15 CA267890-01

PI Name(s) CHOY, JOHN SING

Title Characterizing Regulatory Mechanisms Underlying Drug Resistance in Breast Cancer Using Keratin 19

Institution CATHOLIC UNIVERSITY OF AMERICA

Abstract Drug resistance continues to be the major limiting factor in achieving cures for cancer patients. In breast cancer, cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors have been approved recently to treat patients with advanced estrogen receptor-positive tumors. However, most patients exhibit resistance due to a lack of predictive biomarker. Understanding the molecular basis underlying drug resistance is required to provide a critical breakthrough in identifying a predictive biomarker and developing effective therapeutic strategies. Based on our previous work and preliminary data, we propose that a cytoskeletal protein keratin 19 (K19), which currently serves as one of the most reliable diagnostic and prognostic markers, regulates signaling events to impact resistance against CDK4/6 inhibitors. We found that K19 binds to and inhibits a multifunctional kinase GSK to stabilize a CDK4/6 partner cyclin D3 in breast cancer cells. K19-GSK3 interaction was associated with decreased accumulation of GSK3 in the nucleus that is crucial to GSK3 function. Moreover, K19 promoted proliferation and maintained the sensitivity of cells to CDK4/6 inhibitors. We hypothesize that cyclin D3 stabilized by K19-dependent inhibition of GSK3 causes tumors to become dependent on the CDK4/6 pathway for growth and sensitizes them to CDK4/6 inhibitors. At the molecular level, we surmise that K19 filaments serve as cytoplasmic scaffolds for GSK3 to prevent its entry into the nucleus where it phosphorylates cyclin D3 for degradation. To address our hypotheses, we propose to 1) determine K19 effects on the GSK3 signaling network, 2) characterize the interaction between K19 and GSK3, and 3) determine the impact of K19 on drug resistance and tumor growth. To this end, in Aim 1, we will identify upstream regulators of K19-GSK3 interaction as well as GSK3 targets whose activities affect K19- dependent phenotypes. In Aim 2, GSK3 -binding deficient K19 mutant will be characterized to determine how K19 interacts with GSK3 and affects the nuclear entry of GSK3 . In Aim 3, we will assess how K19 affects sensitivities of various breast cancer subtypes to CDK4/6 inhibitors and test the role of K19 and GSK3 on the sensitivity of tumors to a CDK4/6 inhibitor in vivo. Upon successful completion, the knowledge gained from the study can help design new drugs targeting K19-dependent signaling pathways, establish K19 as a predictive biomarker for response to CDK4/6 inhibitors, and develop effective therapeutic strategies to combat drug resistance.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant 1 R15 CA264735-01

PI Name(s) CRAWLEY, WILLIAM SCOTT

Title CDHR5 tumor suppressor function in the intestine

Institution UNIVERSITY OF TOLEDO

Abstract Cadherin related family member 5 (CDHR5) is a type 1 transmembrane adhesion molecule that is highly expressed in the gut epithelium, but is almost always lost in colorectal cancer (CRC). The promising CRC chemopreventive drug 5-ASA (mesalazine) potently upregulates CDHR5 expression as part of its chemopreventive activity, and direct overexpression of CDHR5 reduces the tumor formation potential of CRC cells that have been injected into mice. Together, these results implicate CDHR5 as a tumor suppressor in the gut. Despite this, the functional properties of CDHR5 that allow it to act as a tumor suppressor have not been defined. This represents a significant gap in knowledge of how CDHR5 acts as a barrier against CRC. This knowledge gap prevents the development of chemopreventive CRC therapies involving upregulation of CDHR5, as seen with the drug 5-ASA. The long-term goal of this study is to understand the role of the adhesion molecule CDHR5 in the intestine. The current objective of this proposal is to directly investigate the properties of CDHR5 that allow it to suppress CRC in the gut. Preliminary studies discovered that CDHR5 localizes to the distal tips of microvilli that cover the apical surface of intestinal epithelial cells. Here, CDHR5 forms a strong extracellular interaction with another cadherin, CDHR2, to physically link neighboring microvilli together, organizing them into an ordered array known as the intestinal brush border. Overexpression of CDHR5 results in a striking effect on CRC cells, with cells polarizing to form an increased density of apical microvilli that are longer in length. This shift towards a 'hyper-polarized' state is partially dependent upon the intracellular cytoplasmic domain of CDHR5. For this proposal, the central hypothesis is that CDHR5 contributes to the integrity of the intestinal epithelium to guard against CRC development, in a manner dependent on CDHR5 cytoplasmic binding partners and its extracellular adhesion activity. This hypothesis will be tested through three specific aims: 1) Determine the functional interplay between the two splice isoforms of CDHR5. This aim will explore the discovery that the two splice isoforms of CDHR5 interact to form a mature functional complex that targets to apical microvilli. 2) Identify factors associated with the cytoplasmic domain of CDHR5. This aim will employ an innovative protein purification approach to directly identify the in vivo binding partners for the cytoplasmic domain of CDHR5. The role of binding partners in CDHR5 function will be explored using knockdown/knockout studies an enterocyte model. 3) Determine the functional properties of CDHR5 required to reduce the tumor formation potential of CRC cells that have been implanted in a nude mouse model. This aim will assess whether the adhesion capacity and/or cytoplasmic binding partners are required for CDHR5 to reduce tumor formation of CRC cells. The approach is innovative, since it challenges the existing viewpoint that CDHR5 functions as a tumor suppressor in the gut by interacting with and sequestering β-catenin. The proposed research is significant because loss of CDHR5 is highly correlated with development of CRC and predicts poor survival of cancer patients.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant	1 R15 CA287338-01
PI Name(s)	CREAMER, BRADLEY ALLEN (contact); STAUDINGER, JEFFREY L
Title	Investigating the Roles of Pregnane X Receptor in Human Breast Cancers
Institution	KANSAS CITY UNIV OF MEDICINE/BIOSCIENCES

Abstract

Breast cancer is the second most common type of cancer diagnosed worldwide and is also the second leading cause of cancer-related deaths among women overall. While much progress has been made in the diagnosis and treatment of breast cancer, traditional chemotherapeutic treatments can lead to the development of chemotherapeutic resistance and remains a major challenge in overall patient outcomes. The activation of the Pregnane X Receptor (PXR) by numerous endo- and xeno-biotics, including several chemotherapeutic drugs, is known to regulate many of the metabolic pathways associated with drug metabolism and resistance. The long-term goal is to understand the transcriptional and metabolic targets of PXR in human breast cancers, and to apply this knowledge in their treatment in order to improve patient outcomes. The objective of this application is to elucidate the effects of PXR expression and activation on the transcriptome and phenotypic changes in human breast cancer cell lines. The central hypothesis, which is formulated on PXRs known roles in drug metabolism in healthy and pathogenic tissues as well as preliminary data, is that PXR activation increases xenobiotic metabolism via the induction of Phase I and II enzymes, as well as Phase III transporters, in addition to alterations in the cell cycle and apoptosis in breast cancer. The rationale for the proposed research is that once the transcriptional and phenotypic alterations in response to PXR activation are identified, pharmacological agents or regimes that interfere with these pathways could be developed to decrease the chemoresistance of breast cancer. The objective of this project will be accomplished by two specific aims: (1) Identify global gene expressions changes in human breast cancers associated with pregnane x receptor expression and/or transcription factor activation. An RNA-seq based approach will be used to identify changes to the transcriptome in response to endogenous and exogenous PXR expression and activation. (2) Examine the effects of PXR expression and activation in models of breast cancer on cell survival and chemoresistance. In- vitro based assays examining the effects of PXR on various aspects of the cell cycle, survival, apoptosis, and invasiveness will be utilized. This study is innovative because it shifts the focus of PXRs roles from normal liver and intestinal drug metabolism in order to fill a large void in our understanding of PXRs influence on breast cancer, and potentially in many other types of cancer as well. The proposed project is significant because it will uncover genes and metabolic pathways that are critical to the development of chemotherapeutic resistance in breast cancer, identifying new potential therapeutic targets.

DCB Branch	Cancer Cell Biology
Program Director	LUO, RUIBAI

Grant 2 R15 CA242351-02

PI Name(s) CUDIC, MARE

Title Mechanistic insight into tumor-associated MUC1 glycopeptides binding to macrophage galactose-type lectin

Institution FLORIDA ATLANTIC UNIVERSITY

Abstract

MUC1, the heavily glycosylated cell-surface mucin, is altered in both expression and glycosylation patterns in human carcinomas of the epithelium. Tumor-specific glycopeptide epitopes of MUC1 are recognized by a variety of lectin receptors on immune cells. These interactions have not been extensively studied, despite the fact that aberrant tumor glycosylation alters how the immune system perceives the tumor and can also induce immunosuppressive signaling leading to the creation of a pro-tumor microenvironment favoring tumor progression and metastasis. In addition, it has recently been proposed that the tumor glycocode may be considered a novel immune checkpoint. In this renewal application, we will continue to examine epitope heterogeneity, the glycoside cluster effect, and the steric hindrance effect of neighboring glycans on binding to lectins (Aim 1). Specifically, we will focus on the design of novel synthetic tools such as a) structurally well- defined MUC1 glycopeptides with varying multivalency and b) MUC1-derived positional scanning synthetic glycopeptide combinatorial library (PS-SGCL) displaying native-like heterogeneous and aberrant O-glycan epitopes, sialylated Tn and Thomsen–Friedenreich (TF) antigens. The thermodynamic profile of the interaction of MUC1- derived glycopeptides with macrophage galactose-specific lectin (hMGL) and Siglecs (-7, -9 and/or - 15) will be assessed by ITC. The binding kinetics will be determined by direct
measurement of the strength of unbinding of receptor-ligand interactions by AFM (Aim 2). We are particularly interested in how protein scaffold contributes to the interactions with lectins and also the role of water in protein hydration and the binding complex formation with the ligand. All these are known variables that may impact the receptor targeting efficacy and immune response induced by immune cells. Furthermore, this renewal application proposes to use MUC1 glycopeptides and PS-SGCLs in the discovery and assessment of binding specificities of anti-MUC1 antibodies found in sera from cancer patients with MUC1+ tumors (Aim 3). We are focusing on the identification of a new class of antibodies targeting ""dynamic glycopeptide neoepitopes"". In addition to the research aims, we will continue to recruit the next generation of scientists into the glycoscience field by offering exciting research opportunities for undergraduate and graduate students at the interface of chemistry and immunology. In summary, the proposed study will facilitate a deeper understanding of the principles of glycan-protein interactions and general rules that govern the ability of lectins to regulate immune response by engaging glycoproteins on the cell surface. This may constitute an important roadmap for translating fundamental MUC1 glycobiology knowledge toward next-generation cancer immunotherapies.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director AMIN, ANOWARUL

Grant 1 R15 CA274384-01A1

PI Name(s) DAVIE, JUDITH KIMBERLY

Title Characterization of the Regulation and Gene Targets of TBX2 in Rhabdomyosarcoma

Institution SOUTHERN ILLINOIS UNIVERSITY CARBONDALE

Abstract My lab is focused on understanding the molecular events that result in the transition of a skeletal muscle precursor cell to a rhabdomyosarcoma (RMS) tumor cell in hopes to suggest therapeutic strategies to reduce the oncogenic potential of this childhood cancer. We have discovered that TBX2, a T-box family member, is a novel oncogene in RMS tumor cells. TBX2 is often over expressed in cancer cells and is thought to function in bypassing cell growth control by the repression of p14ARF and p21. The cell cycle regulator p21 is required for the terminal differentiation of skeletal muscle cells and is silenced in RMS cells. We have found that TBX2 represses p21, p14ARF and the tumor suppressor PTEN in RMS cells and inhibits the activity of the myogenic regulatory factors through binding to MYOG and MYOD1. Thus, TBX2 both promotes proliferation and represses terminal differentiation. Given the crucial importance of TBX2 in driving tumor cell proliferation in RMS, it is important to understand the regulation of TBX2. In other cells, TBX2 is regulated by PAX3, a paired- box transcription factor essential in skeletal muscle progenitor cells. RMS cells are characterized by expression of PAX3 and the more aggressive subtype of RMS contains a translocation that fuses PAX3 with the transactivation domain of a forkhead transcription factor producing a PAX3-FOXO1 fusion protein. FGF signaling has been shown to activate both PAX3 and TBX2 in other systems, suggesting that this may occur in skeletal muscle as well. We have also found that the highly related T-Box factor, TBX3, represses TBX2 in normal skeletal muscle and RMS, but TBX3 is itself silenced by the polycomb repressive complex (PRC2) in RMS. Our data suggest that TBX2 is part of the normal regulatory program expressed in proliferating myoblasts which is silenced in differentiated cells during development. The goal of this proposal is to investigate the regulation and additional gene targets of TBX2 in RMS cells, which will provide the needed molecular insight to potentially harness the expression or function of TBX2 therapeutically. In our first aim, we will identify mechanisms regulating TBX2 expression by determining if PAX3/PAX-FOXO1 and the FGF signaling pathway activate TBX2. In the second aim, we will characterize novel tumor suppressor genes regulated by TBX2 including TP53 and TCEAL7. P53 is a well known tumor suppressor that has been extensively studied in RMS, but our data are the first to implicate TBX2 in the silencing of TP53 in any system and suggest that TP53 is transcriptionally silenced in ARMS. The function of TCEAL7 is unexplored in RMS and may represent a novel therapeutic target. Finally, we will profile TBX2 binding on the genome to identify all genes bound by TBX2 in RMS. Taken together, this work will provide essential insight into the role and regulation of the novel oncogene TBX2 in RMS cells, thus providing a novel therapeutic target for RMS. Understanding abnormalities in gene regulatory pathways is crucial for understanding the pathology of cancer cells and for designing effective therapeutic strategies to improve treatment of rhabdomyosarcoma.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant 1 R15 CA267923-01

PI Name(s) DIAKONOVA, MARIA

Title Role of prolactin in adipocyte-breast cancer cell crosstalk

Institution UNIVERSITY OF TOLEDO

Abstract There is a gap in our understanding of how hormone prolactin (PRL), secreted by adipocytes, and the serine/threonine PAK1 connect to coordinately regulate progression of human breast cancer (BC) and adipocyte-breast cancer cell crosstalk. The long-term goal of this research is to establish PAK1, activated by adipocyte-secreted PRL, as a new diagnostic tool for the metastatic potential of BC cells and provide needed insight into the underlying mechanisms. The overall objective of the current application is to establish an experimental system to study effects of adipocyte-secreted PRL on breast cancer cells and adipocyte-breast cancer cells crosstalk and further establish the mechanisms by which PRL-activated PAK1 contributes to breast cancer. The central hypothesis is that that adipocyte-produced prolactin enhances invasiveness, tumorigenicity and metastatic potential of breast cancer cells through activation of PAK1 kinase and pTyr- PAK1 is a biomarker to predict metastatic potential of these breast cancer cells. The rational for the proposed research is that breast adipocytes secret adipocytokines including PRL, which may influence breast tumor behavior. PRL promotes JAK2 activation and tyrosyl phosphorylation of PAK1 in breast cancer cells. The central hypothesis will be tested by pursuing the following specific aims: Specific Aim 1 will test our working hypothesis that PAK1 activated by adipocyte-derived PRL enhances breast cancer cell invasiveness and cell motility via Tec kinase and that adipocyte-breast cancer cells crosstalk induces secretion of MMP-11 by adipocytes and MMP-1 by breast cancer cells. Specific Aim 2 will test our working hypothesis that PAK1 activated by adipocyte-derived PRL affects tumorigenicity and metastatic potential of breast cancer cells in vivo and that pTyr-PAK1 can serve as a biomarker to predict breast tumor metastasis. Under the sub-aim, characterized PAK1 phospho-specific antibodies will be used to evaluate metastatic potential of the cells at an early stage of BC. This proposal is conceptually innovative because it is expected to vertically advance and expand our understanding of how obesity through adipocyte-secreted PRL influences BC and the unique role of PRL-activated PAK1 in this process. This proposal is technically innovative as well because it will employ unique PAK1 phospho-specific antibodies. The proposed research is significant because the elucidation of PRL/PAK1 mediated intracellular signaling pathways will enhance our understanding of the mechanisms involved in regulation of BC and will lead to development of PAK1-based tools for BC diagnosis.

DCB Branch Tumor Metastasis

Program Director GRIL, BRUNILDE M

Grant 1 R15 CA249653-01

PI Name(s) DU, LIQIN

Title Molecular and therapeutic mechanisms of differentiation-inducing microRNA miR-506-3p in neuroblastoma

Institution TEXAS STATE UNIVERSITY

Abstract There is a lack of understanding how critical microRNAs (miRNAs) are in controlling neuroblastoma (NBL) cell differentiation. This prevents the application of miRNA-based therapeutics to NBL differentiation therapy, which is an approach to induce malignant cells into terminal differentiation and thereby block tumor growth. The long- term goal of the applicant is to define the role of miRNAs in regulating NBL cell differentiation and make contributions to the development of miRNA-based differentiation therapy for NBL. The objective of this study is to elucidate the mechanisms underlying the differentiation-inducing function of a differentiation-inducing miRNA recently identified in our group, miR-506-3p, and to develop miR-506-3p analogs with enhanced differentiation-inducing activity. The central hypothesis is that miR-506-3p functions as a inducer of cell differentiation through directly targeting a group of genes that play key roles in regulating NBL cell differentiation, and that miR-506-3 analogs with enhanced differentiation-inducing activity can be developed by modifying the nucleotide sequence in the non-seed region of the wildtype miR-506-3p. This hypothesis is supported by strong preliminary data generated in the applicant's lab. The following Specific Aims are proposed: Aim 1, Identify novel miR-506-3p targets that mediate its differentiation-inducing function. A functional high-content screening (HCS) approach will be used to systematically investigate its targets regarding their role in regulating NBL cell differentiation. The direct interactions of miR-506-3p with the targets identified from screen will be validated by combining a biotinylated-miRNA pull-down assay and a luciferase reporter assay. Since the molecular mechanisms of regulating NBL cell differentiation are still poorly understood, we expect that a comprehensive investigation of the miR-506-3p targets will reveal genes that were previously unknown to regulate NBL differentiation. Aim 2, Develop novel miR-506-3p analogs with enhanced differentiation-inducing activity. Synthetic analogs of miR-506-3p will be designed, and analogs with significantly increased differentiation-inducing activity relative to wildtype miR-506-3p mimic will be identified using HCS and further in vitro validation analysis. The identified analogs will then be preliminarily evaluated for their therapeutic potential by examining their generic differentiation-inducing activity in a panel of NBL cell lines with diverse genetic background, and by examining their effect on viability of non-NBL cells in order to select analogs with minimum non-specific cytotoxicity. The study is innovative because it will elucidate a novel cell differentiation pathway in NBL mediated by miR-506-3p and its target genes, and it will identify novel miRNA- 506-3p analogs that has potential to be developed as differentiation therapeutic agents. The study is significant because it is expected to advance the understanding on the mechanisms of NBL cell differentiation, and to pave the way to develop more effective miRNA-based differentiation therapies for treating NBL, which is expected to eventually benefit the survival and wellness of NBL patients.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant 1 R15 CA246419-01A1

PI Name(s) FERRAN, MAUREEN C

Title NFkB-dependent antiviral pathways in VSV-resistant cancer cells

Institution ROCHESTER INSTITUTE OF TECHNOLOGY

Abstract Antiviral responses are defective in many human tumors, leaving them susceptible to infection by "oncolytic" viruses such as vesicular stomatitis virus (VSV). In contrast, normal cells are not infected because they mount an innate immune response. Studies show that some cancers are resistant to VSV infection because they retain these antiviral responses. For example, many VSV-resistant prostate cell lines have constitutively active NFκB, while VSV-sensitive prostate cancer cell lines do not. Therefore it is important to delineate the mechanisms of sensitivity versus resistance of cancers to VSV. The wild-type M protein inhibits NFκB activation, the IFN response, and host gene expression, but different M protein mutations can selectively eliminate each of these functions. These findings have led us to conclude that the M protein uses at least two mechanisms to limit expression of antiviral genes: M-mediated inhibition of global host transcription (the first suppressor) and inhibition of NFκB activation (the second suppressor). Our preliminary in vitro and modeling data support our central hypothesis that VSV uses multiple strategies to control antiviral gene expression in response to VSV infection, including global host transcription inhibition, targeting of steps upstream of IKK in the RIG-I pathway, and suppression of antiviral genes controlled by NFκB. The objectives of this study are to enhance our understanding of the balance between the host's ability to activate an NFκB-dependent antiviral response and the virus's ability to evade these defenses; and how this impacts the use of oncolytic viruses to treat tumors that constitutively express antiviral genes. The goal of this study is to determine the effects of M protein mutations on NFκB-dependent responses in VSV-sensitive (LNCaP) versus VSV-resistant (PC3) prostate cancer cell lines using the innovative combination of in vitro and in silico modeling studies. In Aim 1, we will determine NFκB activation and expression of NFκB- dependent antiviral genes (e.g. interferon, IL-6 and TNF-α) in LNCaP and PC3 cells infected with viruses bearing different mutations in the M protein (Aim 1A). To determine the role of NFκB-dependent pathway activation in resistance to VSV, the transcriptomes of infected LNCaP and PC3 cells will be compared by RNA- seq (Aim 1B). We have developed an executable network model of the intracellular signaling pathways impacted by wildtype and M protein mutant VSV in mouse cells. We will tune this network using data specific to the context of VSV infection of human prostate cancer cell lines (generated in Aim 1) and perform simulations to identify key NFκB-dependent signaling molecules and interactions responsible for VSV sensitivity or resistance in prostate cancer cells (Aim 2A). Finally, new in vitro experiments will be performed to validate these predictions (Aim 2B). In addition to these scientific merits, this project will provide undergraduate and Master's students with a quality biomedical research experience, foster collaborations, and significantly enhance the research environment at The Rochester Institute of Technology.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant 2 R15 CA235749-02

PI Name(s) GE, XUECAI

Title Regulation of Hedgehog Signaling by new cilium proteins

Institution UNIVERSITY OF CALIFORNIA, MERCED

Abstract Hedgehog (Hh) signaling is widely involved in development and cancers, including medulloblastoma (MB), the malignant pediatric brain tumor. Current treatment regimens bring life-long devastating side effects to survivors, generating a huge burden to the patients' family. The current Hh pathway inhibitors are challenged by drug resistance and tumor relapse. We aim to understand the basic transduction mechanisms of the Hh pathway in order to provide new clues to treat Hh-related cancers. This proposal is based on my previous discovery that PDE4D controls local PKA activity at specific subcellular sites to regulate Hh signaling. In the past two years of this grant, we have studied the control of Hh signaling by local PDE4D-PKA at the centrosome. We found that 1) PDE4D3 is anchored to the centrosome by myomegalin to locally inhibit PKA activity, 2) knocking out myomegalin with shRNA or CRISPR/Cas9 specifically increases PKA activity at the centrosome, and 3) this selectively inhibits Hh signaling without interfering other PKA-related cellular events. Based on the exciting results and the innovative tools we have built, in the current proposal we will expand our research to systemically investigate the regulation of Hh signaling by local PKA in the cilium. We will 1) define the signaling mechanism from Smo to PKA in the cilium, a mysterious step in the Hh pathway; 2) determine the effects of selectively promoting ciliary PKA on Hh signaling and Hh-related tumor growth; and 3) identify the signaling transducers associated with Smo via proximity labeling-based proteomics to thoroughly illuminate its downstream signaling cascade during Hh transduction. Our approach is innovative because it combines cutting edge techniques to elucidate the long-standing questions in the Hh pathway. Its significance is underlined by the expectation that it will highlight new approaches to treat pediatric brain tumor with fewer side effects. Equally importantly, this research funding will allow me to engage first generation college students in biomedical research at UC Merced to diversify and strengthen academic research in the economically deprived California Central valley.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant 1 R15 CA267892-01A1

PI Name(s) HARROD, ROBERT L

Title Roles of p53-Regulated Pro-Survival Signals in Carcinogenesis by HTLV-1 and High-Risk Subtype HPVs

Institution SOUTHERN METHODIST UNIVERSITY

Abstract The human T-cell leukemia virus type-1 (HTLV-1) is a delta oncoretrovirus that infects and transforms CD4+ T-cells and causes adult T-cell leukemia/lymphoma (ATLL), an aggressive hematological malignancy that is generally resistant to conventional anticancer therapies. The 3' end of the HTLV-1 genome encodes several regulatory proteins (i.e., Tax, Rex, HBZ, p8I, p12I, p13II, and p30II) from a highly- conserved nucleotide sequence, known as pX, which is retained in the majority of ATLL clinical isolates. For nearly four decades, the HTLV-1 has been extensively studied as a general informative model for viral carcinogenesis; however, to date, none of its products have been shown to contain structural or functional similarities to other oncogenic viruses beyond the primate T-cell lymphotropic virus (PTLV) family. My laboratory has identified a core structural domain within the HTLV-1 p30II protein with homology to the E6 oncoproteins of high-risk subtype human papillomaviruses (hrHPVs). Both HTLV-1 p30II and HPV E6 cooperate with the cellular oncoprotein, c-Myc, and prevent p53-dependent apoptosis by inhibiting TIP60-mediated acetylation of the p53 protein on lysine residue K120. Intriguingly, p53 is rarely mutated in HTLV-1+ ATLL and HPV+ cervical cancer clinical isolates –although E6 degrades the p53 protein and significantly reduces its expression through interactions with the ubiquitin ligase, E6AP. Our preliminary studies have demonstrated that the HTLV-1 p30II and HPV16/18 E6 viral oncoproteins induce the expression and mitochondrial localization of the TP53-induced glycolysis and apoptosis regulator (TIGAR) and p53-inducible ribonucleotide reductase (p53R2). Primary patient-derived ATLL and HPV+ cervical carcinoma samples contain elevated levels of TIGAR and p53R2 that correlate with oncogenic c-Myc expression. We have further shown that lentiviral-siRNA-knockdown of TIGAR inhibits in vivo tumorigenesis and metastatic disease progression in xenograft models of HTLV-1-induced T-cell lymphoma and HPV-induced squamous cell carcinoma. Based upon these findings, we hypothesize that the unrelated HTLV-1 and high-risk subtype HPVs may have evolved similar strategies to deregulate host oncogenic and pro-survival signaling pathways by targeting p53 functions. The following Specific Aims are proposed for this R15 AREA project: 1) to elucidate the molecular mechanisms by which the HTLV-1 p30II and high-risk HPV E6 oncoproteins modulate p53-regulated pro-survival signals, 2) to determine how TIGAR and p53R2 contribute to the cooperation between HTLV-1 p30II or hrHPV E6 oncoproteins and cellular oncogenes, and 3) to elucidate the roles of these p53-regulated pro-survival signals in HTLV-1 and HPV-induced tumorigenesis in vivo. The proposed studies will yield valuable new insight into the evolutionary relationship between HTLV-1 and other cancer-inducing viruses and advance our understanding of the roles of p53-regulated pro-survival signals in viral carcinogenesis.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant	1 R15 CA242344-01A1
PI Name(s)	JASMIN, JEAN-FRANCOIS; MERCIER, ISABELLE (contact)
Title	CAPER, a New Regulator of DNA Damage and Repair in Triple Negative Breast Cancer
Institution	SAINT JOSEPH'S UNIVERSITY

Abstract

Triple negative breast cancer (TNBC) has the poorest clinical outcome amongst all breast cancer subtypes. TNBC lacks the expression of the three major receptors found in other subtypes (estrogen receptor [ER], progesterone receptor, and/or hormone epidermal growth factor receptor-2) making this cancer particularly challenging with regards to treatment modalities. Novel targeted treatments that could kill TNBC cells or sensitize them to chemo- and radiation therapies are highly coveted to increase survival of these patients. Due to its fast proliferative rate, TNBC relies heavily on DNA repair mechanisms for its survival and proteins involved in this important checkpoint are attractive targets for cancer treatment. CAPER (Rbm39) protein was recently shown to be overexpressed in breast cancer specimens compared to normal breast tissues. While CAPER knockdown inhibits breast cancer cell growth, its role on DNA damage and repair mechanisms in breast cancer and its role in TNBC progression and response to chemo- and radiation therapies remain completely unexplored. Our preliminary data demonstrate that knockdown of CAPER expression in TNBC cells increases DNA damage as reflected by increased phosphorylation of H2AX and ATM. The decreased total cell number and increased caspase-3/7 cleavage observed following CAPER knockdown in MDA-MB-231 and BT549 TNBC cells is suggestive of insurmountable DNA damage leading to programmed cell death (apoptosis). The effect of CAPER knockdown on DNA damage in TNBC cells was cell cycle-independent and selective to cancer cells, as non- tumorigenic cells lack the expression of CAPER and remain unaffected following delivery of lentiviral CAPER shRNAs. Our preliminary results also revealed that DNA repair proteins RAD-51, C-Alb and RB were significantly downregulated in TNBC following CAPER knockdown. We posit that CAPER overexpression in TNBC plays an important role in protection against DNA damage by optimizing DNA repair pathways. The current proposal builds on our previous work and preliminary results and aims to delineate the roles of CAPER in basal DNA damage/repair pathways and to determine its clinical relevance in TNBC growth using both immunocompromised (xenograft) and immunocompetent (syngeneic) orthotopic mouse models (Aim 1). Importantly, we will also use these TNBC cells and mouse models to test the unexplored role of CAPER in the response to DNA damaging chemo- and radiation therapies in both in vitro and in vivo settings (Aim 2). The current proposal will validate the role of CAPER as an important signaling molecule in the progression of TNBC as well as response to DNA damage using clinically relevant models that also incorporate immune surveillance and will be insightful in the further development of targeted therapies for the treatment of TNBC.

DCB Branch	DNA and Chromosome Aberrations
Program Director	WITKIN, KEREN L

Grant	1 R15 CA271221-01A1
PI Name(s)	JIN, KIDEOK
Title	Targeting secreted factors in endocrine resistant breast cancer therapy
Institution	ALBANY COLLEGE OF PHARMACY

Abstract

Various types of endocrine therapy have been used for postmenopausal women with early stage breast cancer. These therapies are safe and effective, but initially responsive breast tumors often develop resistance and eventually recur. Since a communication between tumor and tumor circumstance plays an important role to grow tumor and spread tumor to secondary organs, we examined the communication of endocrine resistant breast cancer with stroma which is the part of a tissue or organ that has a connective and structural role in cancer. We established four different endocrine resistant breast cancer cell lines and examined the communication of these cells with four stromal cells. We found that CCL5 and endoglin might play an important role in endocrine resistant breast cancer. Thus, we will 1) Investigate the role of CCL5 and endoglin in the crosstalk between ERBC cells and stromal cells, 2) Unveil the mechanism of the upregulated CCL5 and endoglin in crosstalk between ERBC and stroma, and 3) Develop therapeutic strategies to block the paracrine interaction between the stromal cell and ERBC cell. We will investigate which secreted factors could serve as a key regulator in the transition of breast cancer cells to endocrine resistance and gaining of an aggressive phenotype. Further, secreted factors from the communication between endocrine resistant breast cancer and stroma might prove to be an attractive target for breast cancer drugs. Most of the cancer drugs in use currently have been developed to directly impact tumor cells. These drugs do not do much to block signals coming from tumor circumstance. Our proposed investigation will give us a small list of the most potent of these signals. Drugs, some of which we will identify here, and others that will have to be developed, that block these signals could limit the ability of the tumors to spread to secondary organs, which should result in overall survival gains for patients.

DCB Branch	Tumor Biology and Microenvironment
Program Director	BERA, TAPAN K

Grant 1 R15 CA242471-01A1

PI Name(s) JORCYK, CHERYL LYNN

Title OSM-induced IL-6 and ER status in metastatic breast cancer

Institution BOISE STATE UNIVERSITY

Abstract Despite new drugs and improved detection methods, the five-year survival rate for female breast cancer patients with distant metastases lingers at a dismal 27%. This is in part due to a critical lack of therapeutics specifically targeting triple negative breast cancer (TNBC), which is estrogen receptor-negative (ER-) progesterone receptor- negative (PR-) and HER2-negative (HER2-). This negative receptor status eliminates many therapeutic options that exist for ER+ PR+ HER2+ patients. Our long-term goal is to provide preventative and therapeutic treatment options for patients with metastatic breast cancer by identifying novel targeted interventions against the OSM/OSM receptor (OSMR) axis. The goal of this proposal determine how ER status contributes to OSM- induced IL-6 and OSM-promoted breast tumor invasion and metastasis. OSM is an interleukin-6 (IL-6) family cytokine important in inflammation, which is produced by activated T-cells, monocytes/macrophages, neutrophils, and human breast cancer cells. Our published data demonstrates that OSM induces osteolytic bone metastases in vivo, implicating OSM as an important factor in the localized bone metastatic microenvironment. Published data from our lab and others show that OSM promotes an epithelial-mesenchymal transition, tumor cell detachment, and an invasive phenotype in vitro, suggesting that this cytokine may be a critical factor driving breast cancer invasion and tumor cell dissemination. We have recently shown that OSM increases circulating tumor cell (CTC) numbers and metastases to lung in vivo, consistent with a more general role for OSM in metastasis. For this proposal, we hypothesize that OSM-induced IL-6 expression is dependent on a negative estrogen receptor alpha (ERα) status and that the presence of ERα will decrease OSM-driven breast cancer metastasis. To test our hypothesis, we propose two specific aims: 1) Determine the mechanisms by which ERα represses OSM-induced IL-6 expression, and 2) Analyze OSM and OSM-induced IL-6 and metastatic potential in ER+ and ER- breast cancer models. In the first aim, OSM-induced IL-6 production will be investigated in both parental and genetically modified ER+ and ER- human breast tumor cells, and the mechanisms by which ER represses OSM-induced IL-6 production will be investigated. In the second aim, genetically manipulated ER+ and ER- breast tumor mouse models will be studied with the presence or absence of OSM and Siltuximab, a neutralizing monoclonal antibody against IL-6. Together, these studies will both clarify the complex association between OSM-induced IL-6 and ER status as well as help define the patent population that will most benefit from anti-OSM therapeutics.

DCB Branch Tumor Metastasis

Program Director GRIL, BRUNILDE M

Grant 1 R15 CA246335-01A1

PI Name(s) KHALIL, ANDRE (contact); TILBURY, KARISSA BETH

Title Coupling Advanced Computational Analyses for Mammography and SHG Imaging for Early Detection of Breast Cancer Tissue Microenvironment Disruptions Accompanying Tumorigenesis

Institution UNIVERSITY OF MAINE ORONO

Abstract The breast tumor microenvironment plays a key role in the early development of cancer. An improved understanding of the tumor microenvironment will support the early detection of disease and the development of novel, more effective therapies. Current radiological interpretation of mammograms is based on identifying breast lesions and assessing their potential malignancy and lethality. The biomedical research community has yet to correlate the mammographic signatures of microenvironment alterations and loss of tissue homeostasis accompanying, or even preceding tumorigenesis. The PI recently used a novel and powerful computational technique to demonstrate that tissue disruption and loss of homeostasis can be quantitatively and objectively assessed from standard clinical mammography. These preliminary findings pertain only to single diagnostic mammograms; the proposed AREA project will analyze longitudinal sequences of mammograms over multiple years prior to, and approaching the year of diagnosis, thus revealing the progression of healthy breast tissue microenvironment towards a disrupted state. It is hypothesized that the tissue disorganization signature present in mammographic data is associated with tissue disruption and loss of tissue homeostasis in the microenvironment. Team members will verify this hypothesis by imaging breast tissue samples to characterize, at the cellular level, disruption of collagen structures associated to tumor microenvironment alterations accompanying tumorigenesis. This project's specific aims include: 1) verifying that mammographic tissue disruption accompanies and perhaps even precedes tumor development; 2) developing an in silico model of microcalcification growth into mammographic breast tissue environment, 3) improving the computational efficiency of our data analysis, and 4) linking morphological features of the breast tumor microenvironment imaged by mammography to cellular-scale alterations imaged by second harmonic generation microscopy. The proposed research will advance the education and career preparation of undergraduates within Maine's only biomedical engineering program, nurture a growing partnership among the University of Maine, Spectrum Healthcare Partners and Maine Medical Research Institute, introduce two UMaine faculty to biomedical research, and support the early career development of two assistant professors.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director MILLER, DAVID J

Grant 1 R15 CA267804-01A1

PI Name(s) KIM, CHONGWOO A

Title Molecular Basis of the Selective Assembly of Functionally Distinct PRC1s

Institution MIDWESTERN UNIVERSITY (GLENDALE AZ)

Abstract Polycomb repressive complex 1 (PRC1) is a multi-protein assembly that epigenetically regulates chromatin, which when misregulated, results in cancer. Originally identified in Drosophila as a four-component complex, PRC1 has expanded its membership and functional repertoire over evolution. An important unanswered question concerning human PRC1 is how certain combinations of PRC1 proteins assemble while others do not, and how the types of PRC1s formed change during cellular differentiation. PRC1 assembly is currently understood to occur through a series of 1:1 protein-protein interactions. This view, unfortunately, does not explain how the protein-protein interaction of one PRC1 domain can somehow influence the binding selectivity of another, seemingly unrelated protein-protein interaction. We have identified novel interactions secondary to the known 1:1 direct interactions that provide selective checkpoints for including specific protein combinations thereby allowing selective PRC1 assemblies. OBJECTIVE: We will systematically characterize these secondary protein-protein interactions then investigate its role in the assembly and function of the distinct PRC1s. METHODS: We will use biophysical methods (X-ray crystallography, analytical ultracentrifugation, biolayer interferometry) to dissect the molecular basis of these selective assemblies. The functional consequences of the secondary interactions that form these assemblies will be assessed using a novel FRET- based histone modification assay. SIGNIFICANCE: Gene regulation is performed by many different proteins, all of which work within multi-component systems. Current genomic approaches to investigating gene regulation, while informative, lack a molecular perspective of protein-protein interactions necessary for a complete understanding of these systems. We propose to fill this gap by determining how specific PRC1s assemble and function. By defining the molecular choreography underlying PRC1 assembly, we will provide a new perspective on the regulation and function of these complexes in genome regulation. Our results may also be broadly applicable for understanding other gene regulatory systems, many of which utilize multi- component systems with different homolog combinations.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director AMIN, ANOWARUL

Grant 1 R15 CA287203-01

PI Name(s) KUCHERYAVYKH, LILIA

Title Pyk2 regulates extracellular vesicles release in GBM cells and modulates activation of tumor infiltrating myeloid cells

Institution UNIVERSIDAD CENTRAL DEL CARIBE

Abstract Glioblastoma (GBM) is the most aggressive brain cancer and usually fatal within a year after diagnosis. Current treatment approaches provide only a modest, few months, life extension. Tumor infiltrating myeloid cells (TIM) provide strong impact on tumor growth, dispersal and treatment resistance. The current unmet need in GBM treatment is to address the TIM contribution on tumor growth and treatment resistance in treatment protocols. The goal of the study is to identify molecular targets for the GBM treatment approaches aimed at modulation of tumor immune microenvironment. Our recent studies identified significant up-regulation of Proline-Rich Tyrosine Kinase 2 (Pyk2) in GBM tumors, compared with healthy brain tissue, as well as, a positive correlation between Pyk2 activation in tumor cells and cytokines expression profile of TIM. Additionally, both tumor resection and temozolomide treatment up-regulate Pyk2 in GBM tumors and affect activation state of TIM. This identified Pyk2 as a candidate prognostic marker for the immune state in GBM tumor microenvironment. Preliminary studies in GL261 glioma cells identified that cells, knocked out for Pyk2, do not release the population of EVs with diameter bigger them 600nm and reduce expression of monocyte chemoattractant protein (CCL2), CCL12 and vascular endothelium growth factor (VEGF) in EV fraction, compared with Pyk2WT cells. Correspondingly, increase of CD86+/CD206+ inflammatory myeloid cell and CD8+ lymphocytes populations were found in TIMs, purified from Pyk2KO tumors, compared with Pyk2WT tumors, in GL261/C57Bl/6 mouse glioma implantation model. Based on these findings we hypothesize that Pyk2 is involved in signaling regulation of release of inflammatory cytokines CCL2, CCL12 and CCL5 through the EVs mechanism, leading to modulation of TIM cells polarization. In this study we will utilize mouse glioma implantation model and primary human GBM cell cultures, with high and low levels of Pyk2 expression, coupled with cell biology approaches, to dissect the role of Pyk2 in regulation of biogenesis and cytokines content of glioma cell derived EVs. We will also examine, in mouse and human in vitro models, the modulation of activation state of microglial cells through the glioma-derived EV mechanism. The purpose of the project is to identify mechanisms of interaction between glioma and TIM cells and the role of Pyk2 signaling in this communication. To test our hypothesis, we propose the following specific aims: Specific Aim 1. To investigate the role of Pyk2 in release of cytokines through the EV mechanism in GBM cells. Specific Aim2. To investigate the role of Pyk2 in regulation of actin-related release of EVs populations. Specific Aim3. To assess the role of vesicular Pyk2 in EV internalization by microglial cells.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant	1 R15 CA280818-01A1
PI Name(s)	LONG, WEIWEN
Title	FBXL16 as a novel factor in promoting endocrine therapy resistance and metastasis of ER+ breast cancer
Institution	WRIGHT STATE UNIVERSITY
Abstract	Endocrine therapy (ET) resistance and metastasis are major obstacles for curing patients with advanced ERα+ breast cancer (ER+ BC). Upregulated oncogenic ERα activity plays critical role in advanced progression of ER+ BC. One essential mechanism of regulating ERα signaling is the ubiquitination- dependent proteasomal degradation of ERα and its co-activators such as steroid receptor coactivator 3 (SRC-3, also known as amplified in breast cancer 1). Owing to its direct effect of targeting ERα for degradation, fulvestrant is the only FDA-approved selective estrogen receptor degrader (SERD) as a first line endocrine agent for metastatic and locally advanced breast cancer and a second line drug for advanced metastatic breast cancer that has progressed after tamoxifen or aromatase inhibitor treatment. Unfortunately, either de novo or acquired resistance to fulvestrant occurs in the majority of the patients with advanced ER+ BCs. The molecular mechanism underlying fulvestrant resistance is still largely unknown. The proposed study aims to test an intriguing hypothesis that the F-Box protein FBXL16 stimulates oncogenic ERα activity by upregulating the stability and expression levels of ERα and coactivator SRC-3, thereby promoting breast cancer development, metastatic progression and endocrine therapy (ET) resistance. Two specific aims will be performed. Aim 1 is to determine the roles of FBXL16 in regulating the stability and expression levels of ERα and ERα LBD mutants; Aim 2 is to determine the role of FBXL16 in ER+ breast tumor growth, metastasis and endocrine therapy resistance. By conducting a variety of both in vitro and cell-based assays and in vivo tumor mouse model experiments, we expect to elucidate how FBXL16 upregulates the protein stability of ERα and ERα-LBD mutants and to determine the roles of FBXL16 in breast tumor development, metastasis and ET resistance. Our ultimate goal is to define FXBL16 as a new prognostic marker and/or a therapeutic drug target for treating advanced ER+ BCs, particularly in the patients with either de novo or acquired resistance to SERDs including those with ERα LBD activating mutations.
DCB Branch	Tumor Biology and Microenvironment
Program Director	BERA, TAPAN K

Grant	1 R15 CA252990-01A1
PI Name(s)	LORICO, AURELIO (contact); RAPPA, GERMANA
Title	Nuclear Transport of Extracellular Vesicle Biomaterials
Institution	TOURO UNIVERSITY OF NEVADA

Abstract

Targeting the interaction between cancer and stromal cells in the tumor microenvironment holds great therapeutic potential. Recently, interest in this type of communication has expanded beyond signaling molecules to include extracellular vesicles (EVs), released by cancer cells and taken up by target cells locally or at distance. Cancer EVs contain proteins and nucleic acids responsible for pro-tumorigenic and pro-metastatic effects. Our knowledge of the EV world is in its infancy. What happens to EV cargo molecules once inside the target cells, and how they exert their biological effects is still obscure. Our long-term goal is to understand the intracellular route(s) and subcellular fate of EV content upon internalization and to apply this knowledge to developing novel therapeutic strategies, especially in cancer. We have recently identified a novel EV nuclear pathway in which a tripartite VOR protein complex, containing VAP-A, ORP3 and Rab7, orchestrates translocation and docking of EV-containing late endosomes into nuclear envelope invaginations (NEI) with subsequent nuclear transfer of EV cargo. Preliminary evidence of the biological relevance of this novel nuclear pathway was shown by the finding that ORP3 and VAP-A are required for pro-metastatic morphological changes of non-metastatic colon carcinoma cells induced by EVs from metastatic colon carcinoma cells. Our central hypothesis is that this intracellular pathway mediates many of the effects of EVs, and that interfering with mechanism/s that regulate i) the interaction between late endosomes and nuclear membrane, and ii) the nuclear delivery of the endocytosed EV components will impair the intercellular crosstalk in the cancer microenvironment, and thereby inhibit tumor growth and formation of metastases. We propose to investigate the effects of nuclear delivery of colon cancer-derived EV biomaterials on MSCs and to carefully define the proteins involved in this novel nuclear pathway. This objective will be accomplished by two specific aims: (1) we will investigate whether this nuclear pathway is required for the transformation of MSCs by EVs derived from metastatic colon carcinoma cells, paying particular attention to its impact on MSC proliferation, migration and invasiveness; (2) we will dissect in detail the VOR complex by deletion or mutation of domains and/or motifs reportedly involved in protein-protein and protein-lipid interactions, and by expression of VAP-A, ORP3 and/or Rab7 mutants in cells depleted of the corresponding gene. In addition, we will investigate whether other proteins and nuclear pore components are involved. This study is innovative because (a) is based on our discovery of a novel EV nuclear pathway and (b) the nucleus as a final destination of EV cargo has not yet been thoroughly investigated. The proposed project is significant because it will (a) clarify a novel and poorly characterized nuclear pathway, (b) strengthen the research environment of our Institution and involve medical students in all phases of the proposed research, (c) open the way to innovative therapies for cancer metastatic disease targeting the tumor microenvironment.

DCB Branch	Tumor Biology and Microenvironment
Program Director	WOODHOUSE, ELIZABETH

Grant 2 R15 CA223964-02A1

PI Name(s) LU, YUAN

Title Identification and Characterization of a Endogenous EGFR Regulatory Locus in Xiphophorus Genome

Institution TEXAS STATE UNIVERSITY

Abstract The epidermal growth factor receptor (EGFR) is a leading oncogene firmly associated with many types of cancer. Both anti-EGFR small molecules and monoclonal antibodies have been developed to block its kinase activity for cancer treatment. However, innate and acquired resistance are frequently observed in clinical application. Such observations significantly limit anti-EGFR medicines usage, and also challenge current knowledge of EGFR-driver cancer. Therefore, it is necessary to study the relationship between EGFR and cancer from a different angle, with different research strategy. Xiphophorus maculatus encodes a mutant, autonomous, dysregulated and oncogenic EGFR, named xmrk. However, carcinogenesis is only observed in backcross interspecies hybrid between xmrk positive and xmrk-null Xiphophorus species, or when xmrk is ectopically expressed in non-Xiphophorus model system (e.g., medaka, murine). These suggest that X. maculatus genome also encode a regulator gene that may co-evolved with xmrk and is able to suppressing its oncogenic activity. Therefore, characterizing how the regulator, termed R(Diff) inhibit xmrk, may lead to novel therapeutic strategy in controlling EGFR. Our recent study has defined the R(Diff) tumor regulatory locus to a 101.7 kbp locus on chromosome 5. This small candidate size and low candidate gene number enables functional and mechanistic studies are impractical. Therefore, this proposal is designed to final determine the R(Diff) locus by examining the following aims: Aim 1: We will characterize all expressed but unannotated genetic elements, in addition to the known coding genes, within the newly identified candidate R(Diff) locus, to fully annotate the R(Diff) locus. Aim 2: We will determine the gene/element(s) carrying R(Diff) activity by performing gene knock-out in non-tumor-bearing Xiphophorus fish, tumor-bearing hybrids, and tumor-bearing transgenic medaka. Aim 3: We will profile xmrk and R(Diff) candidates, as well as phenotypes of several Xiphophorus hybrids, to define R(Diff) by association of candidate sequence with varied phenotypes.

DCB Branch DNA and Chromosome Aberrations

Program Director FINGERMAN, IAN M

Grant 1 R15 CA254006-01A1

PI Name(s) MADLAMBAYAN, GERARD JAMES (contact); VILLA DIAZ, LUIS GERARDO

Title The Role of Hematopoietic Stem and Progenitor Cells in Solid Tumor Growth and Response to Radiation Therapy

Institution OAKLAND UNIVERSITY

Abstract A major component of many solid tumors, including lung cancers, are bone marrow (BM)-derived immune cells that migrate to tumors and aid in their continued growth. While the activity of these cells including tumor associated macrophages (TAMs) has been the subject of intense investigation, we recently identified that BM- derived hematopoietic stem and progenitor cells (HSPCs) are also present in growing tumors and can be functionally maintained intratumorally for long periods of time. Interestingly, the numbers of HSPCs present in tumors directly correlates to the eventual regrowth rates of tumors following radiation therapy (RT). The data suggests that HSPCs represent another important cell population involved in tumor biology, however; their mechanism of action is still unclear. Filling this gap in knowledge will add to the ever-changing understanding of tumor biology. The objective of this proposal is to determine how HSPCs are maintained in tumors and how HSPCs promote tumor regrowth post-RT. Our preliminary data support the idea that HSPCs are maintained through interactions of the integrin CD49f and laminins present within the tumor extracellular matrix. In Specific Aim 1, we will show that this interaction is indeed responsible for HSPC maintenance using in vitro and in vivo strategies that block or enhance this interaction followed by analysis of their effects on HSPC functionality. We will also define the intracellular signaling pathways involved in this process with initial studies focusing on focal adhesion kinase (FAK) signaling. These studies will characterize for the first time a tumor specific niche capable of maintaining HSPCs outside of the BM. In Specific Aim 2, we will demonstrate that tumor treatment with RT exacerbates HSPC migration to tumors and concomitantly disrupts the interaction between CD49f and laminin. We will also show that RT produces tumor microenvironments that favor the differentiation of these ‘released’ HSPCs into tumor supportive macrophages (specifically M2 polarized) to aid in tumor recovery. We will also test the effects of blocking the activity of HSPCs on tumor growth and regrowth post-RT. By completing the proposed studies, our long-term goal is to use the knowledge gained to make a significant contribution towards the development of more robust treatment strategies for patients suffering with solid tumor based cancers.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant 1 R15 CA267835-01

PI Name(s) MAITRA, RADHASHREE

Title Autophagy mediated immune stimulation by reovirus in KRAS mutated colorectal cancer

Institution YESHIVA UNIVERSITY

Abstract The impressive success of cancer immune therapy reached its limitations and failed in its efficacy with immune deserted KRAS mutated tumors that constitute 45% of colorectal cancers. These patients have fewer treatment options necessitating the development of effective alternate therapy. Of late reovirus, with a double- stranded RNA genome, showed therapeutic efficacy in an oncogenic KRAS transformed colorectal tumors. Although previously studied for its oncolytic properties reovirus is being increasingly appreciated for its immune stimulation properties. However, its participation in immune stimulation, precisely utilizing two intimately related immune and autophagic modulatory pathways, remains largely unexplored. Preliminary results indicate that reovirus preferentially exploits the immune deserted mutant KRAS tumor microenvironment to successfully support its propagation in conjunction with destruction of the tumor cells. Furthermore, reovirus induces autophagy in KRAS mutated microenvironment. Knowledge gaps: It is crucial to understand the mechanism adopted by reovirus to facilitate the immune enrichment of mutant KRAS driven immune deserted microenvironment in colorectal cancer. The contribution of autophagic pathway in accentuating the process is unclear. Understanding the control mechanism between the autophagy and immune pathways is critical. Project hypothesis: The dual mode of action exerted by reovirus can be successfully translated to the clinic by augmenting the induction of the autophagy pathway. We propose to study the pattern of reovirus mediated induction of the autophagic machinery and determine how the molecular event is translated in improving the immune stimulation characteristics in KRAS mutated colorectal Cancer. In this context we will make use of KRAS mutant and wildtype colorectal cancer cell lines and our newly developed KPC:APC (tamoxifen- inducible KRAS-mutated colorectal cancer) mouse models along with the well-established syngeneic mouse model of colorectal cancer to mechanistically explore the process of crosstalk between the two pathways. Results will improve the understanding of the dynamics of KRAS mutated immune deserted cold tumors in facilitating reovirus to act as an immune stimulator Impact: This project will have dual impact of providing an enriched biomedical science related research environment to the aspiring undergraduate students as well as to directly address the clinical gap faced by the KRAS mutated colorectal cancer patients. Understanding the mechanism of cross talk between the autophagy and immune pathways followed by research supported recommendation of therapeutic induction of autophagy to improve immune therapy responsiveness of KRAS mutated colorectal cancer will be of paradigm clinical implications. In this pursuit we will AIM1: Demonstrate crosstalk between autophagy and immune pathways in reovirus treated KRAS mutant and wildtype colorectal cancer cell lines. AIM2: Examine if autophagy induction promotes reovirus mediated immune stimulation in KRAS mutated cold colorectal tumors in transgenic and syngeneic mouse models.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant	1 R15 CA274603-01
PI Name(s)	MELENDEZ, JUAN ANDRES
Title	Epitranscriptomic control of ROS
Institution	SUNY POLYTECHNIC INSTITUTE

Abstract

Maintenance of the GSH redox cycle is reliant on the activities of selenocysteine-containing GSH metabolizing enzymes which play fundamental roles in chemoprevention. Selenocysteine is the 21st amino acid and does not contain a dedicated codon. Selenocysteine incorporation during translation requires UGA-stop-codon recoding, which uses specifically modified tRNA for accurate decoding. Dynamic changes in tRNA modification are an epitranscriptomic signal because they regulate gene expression post-transcriptionally. We have shown that the stress-induced translation of many selenocysteine containing ROS detoxifying enzymes is dependent on the Alkbh8 tRNA methyltransferase. Alkbh8 enzymatically methylates the uridine wobble base on tRNASelenocysteine to promote UGA-stop codon decoding. We have developed an Alkbh8 deficient mouse and have used molecular, biochemical, and genomic approaches to demonstrate that Alkbh8Def mouse embryonic fibroblasts (MEFs) and some organs display markers of senescence and a senescence gene signature. Using human cells and our new Alkbh8Def/p16-3MR mice we propose to test the hypothesis that senescence occurs in vitro and in vivo because of defective epitranscriptomic signals that controls selenocysteine utilization. To achieve this two aims will: 1. determine if Alkbh8 and other epitranscriptomic writers that limit selenocysteine utilization restrict the senescence program and 2. determine if Alkbh8-deficiency drives senescence in vivo and whether senescence ablation accelerates or tempers pathologies that accompany selenoprotein loss. Our proposal is being submitted with significant preliminary data supporting the idea that Alkbh8 and epitranscriptomic signals are key to chemoprevention by limiting senescent activity.

DCB Branch	Cancer Cell Biology
Program Director	LUO, RUIBAI

Grant 1 R15 CA242344-01A1

PI Name(s) MERCIER, ISABELLE (contact); JASMIN, JEAN-FRANCOIS

Title CAPER, a New Regulator of DNA Damage and Repair in Triple Negative Breast Cancer

Institution SAINT JOSEPH'S UNIVERSITY

Abstract Triple negative breast cancer (TNBC) has the poorest clinical outcome amongst all breast cancer subtypes. TNBC lacks the expression of the three major receptors found in other subtypes (estrogen receptor [ER], progesterone receptor, and/or hormone epidermal growth factor receptor-2) making this cancer particularly challenging with regards to treatment modalities. Novel targeted treatments that could kill TNBC cells or sensitize them to chemo- and radiation therapies are highly coveted to increase survival of these patients. Due to its fast proliferative rate, TNBC relies heavily on DNA repair mechanisms for its survival and proteins involved in this important checkpoint are attractive targets for cancer treatment. CAPER (Rbm39) protein was recently shown to be overexpressed in breast cancer specimens compared to normal breast tissues. While CAPER knockdown inhibits breast cancer cell growth, its role on DNA damage and repair mechanisms in breast cancer and its role in TNBC progression and response to chemo- and radiation therapies remain completely unexplored. Our preliminary data demonstrate that knockdown of CAPER expression in TNBC cells increases DNA damage as reflected by increased phosphorylation of H2AX and ATM. The decreased total cell number and increased caspase-3/7 cleavage observed following CAPER knockdown in MDA-MB-231 and BT549 TNBC cells is suggestive of insurmountable DNA damage leading to programmed cell death (apoptosis). The effect of CAPER knockdown on DNA damage in TNBC cells was cell cycle-independent and selective to cancer cells, as non- tumorigenic cells lack the expression of CAPER and remain unaffected following delivery of lentiviral CAPER shRNAs. Our preliminary results also revealed that DNA repair proteins RAD-51, C-Alb and RB were significantly downregulated in TNBC following CAPER knockdown. We posit that CAPER overexpression in TNBC plays an important role in protection against DNA damage by optimizing DNA repair pathways. The current proposal builds on our previous work and preliminary results and aims to delineate the roles of CAPER in basal DNA damage/repair pathways and to determine its clinical relevance in TNBC growth using both immunocompromised (xenograft) and immunocompetent (syngeneic) orthotopic mouse models (Aim 1). Importantly, we will also use these TNBC cells and mouse models to test the unexplored role of CAPER in the response to DNA damaging chemo- and radiation therapies in both in vitro and in vivo settings (Aim 2). The current proposal will validate the role of CAPER as an important signaling molecule in the progression of TNBC as well as response to DNA damage using clinically relevant models that also incorporate immune surveillance and will be insightful in the further development of targeted therapies for the treatment of TNBC.

DCB Branch DNA and Chromosome Aberrations

Program Director WITKIN, KEREN L

Grant	1 R15 CA263826-01
PI Name(s)	MILLET, LARRY J
Title	Application of autobioluminescence toward continuous and real-time in vitro/in vivo pre-clinical brain imaging for disease therapeutics
Institution	UNIVERSITY OF TENNESSEE KNOXVILLE

Abstract

This project proposes to develop autonomously bioluminescent (autobioluminescent) patient-derived glioblastoma (GBM) cellular and rodent models capable of substrate-free, continuous, and noninvasive assessment of therapeutic efficacy to enable effective and efficient translational GBM research. GBM is one of the most lethal of human cancers, with less than 3% of patients surviving beyond a five-year period. To assist in the battle against GBM, bioluminescent imaging technologies that facilitate the noninvasive and longitudinal visualization of tumor dynamics have served as valuable tools in translational efforts to better understand the molecular mechanisms of GBM progression and the evaluation of novel therapeutics in pre- clinical small animal models. However, existing bioluminescent imaging approaches that rely upon conventional luciferase reporter systems (firefly, Gaussia, Renilla, etc.) are handicapped for brain imaging studies because they require that the animal subject be injected with a light-activating substrate prior to each and every measurement. For brain imaging in particular, the extraneous addition of this chemical substrate confounds imaging endpoints because its biodistribution and bioavailability is interfered with by the blood-brain barrier and the brain's efflux pumping mechanisms. Although research is being dedicated toward the synthesis of novel bioluminescent reaction substrates with improved access to the brain environment, we have engaged in an entirely different approach by eliminating the need to add substrate altogether. Our technology leverages the development of a synthetic luciferase (lux) cassette that efficiently expresses bioluminescence in mammalian cells independent of any extraneous addition of a light-activating substrate. These cells are able to self-synthesize all of the requisite substrates from intracellular endogenous metabolites and are therefore capable of self- generating 'autobioluminescent' signals under both constitutive and inducible genetic controls. Within the brain environment, such cells go beyond conventional bioluminescent imaging to ultimately enable continuous, noninvasive, and authentic real-time visualization of neurobiological processes. In this research effort, we propose to express autobioluminescence in patient-derived GBM cell lines and validate their application potential in high-throughput in vitro drug discovery assays and in in vivo rodent models. We will specifically develop and characterize 2D, 3D, and 3D astrocyte co-culture assays, create signaling pathway-specific autobioluminescent cellular models for targeted small molecule screening, and establish an orthotopic autobioluminescent xenograft mouse model for in vivo evaluation of chemotherapeutics that we will test in both conscious and anesthetized subjects. The innovative autobioluminescent cellular and animal models developed in this project will improve the status quo of glioblastoma drug screening and testing and facilitate the development of novel glioblastoma therapeutics within a research environment designed to intellectually stimulate and challenge undergraduate student researchers.

DCB Branch	Biophysics, Bioengineering, and Computational Sciences
Program Director	LI, JERRY

Grant 1 R15 CA283775-01

PI Name(s) NESMELOVA, IRINA V

Title The biological effect and the mode of action of the CXCL7-CXCL12 chemokine heterodimer

Institution UNIVERSITY OF NORTH CAROLINA CHARLOTTE

Abstract Chemokines are essential in health and disease such as infections, autoimmune diseases, atherosclerosis, HIV/AIDS, and cancer. Many chemokines engage in heterophilic interactions with each other to form heterodimers, leading to synergistic activity enhancement or reduction dependent on the nature of heterodimer- forming chemokines. The mode of action of chemokine heterodimers remains poorly understood. Previously, we identified all platelet-derived chemokines interacting with the two most abundant platelet chemokines, CXCL4 and CXCL7. The CXCL12 chemokine demonstrated strong heterodimerization with CXCL7. In the current project, we will determine the biological consequences of these new interactions and the mode of action of the CXCL7-CXCL12 heterodimer. In aim 1, we will determine the effect of CXCL7-CXCL12 heterodimerization on CXCL12-mediated activities, that the CXCL7-CXCL12 heterodimer binds and activates the CXCL12's receptor CXCR4, and the molecular mechanism underlying the effect of the CXCL12-based heterodimers on cell migration. In aim 2, we will determine the effect of CXCL7-CXCL12 heterodimerization on CXCL7-mediated activities (neutrophil adhesion and migration) and the involvement of the CXCR2 receptor in CXCL7-CXCL12 heterodimer-mediated activities. The results of this project will deepen our understanding of chemokine biology and inform the future development of novel therapeutic intervention strategies exploiting heterophilic chemokine interactions.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director AMIN, ANOWARUL

Grant 1 R15 CA235436-01A1

PI Name(s) PATHANIA, SHAILJA

Title Base Excision Repair Deficiency as a Risk Modifier in BRCA2 Associated Cancer

Institution UNIVERSITY OF MASSACHUSETTS BOSTON

Abstract Germline mutations in BRCA2 confer exceptionally high risk for breast, ovarian and multiple other cancers. There is strong epidemiological evidence that these risks are modified by genetic factors, however, currently there is no clear understanding of which genetic factors modify the risk of cancer in BRCA2 mutation carriers. An insight into the early driver events will be critical to help design more effective therapeutic, and importantly, preventive strategies for individuals with mutations in BRCA2. In the project outlined here, we will be investigating one such genetic factor that can influence cancer predisposition and/or response to chemotherapeutics in BRCA2 mutation carriers. We have identified members of base excision repair (BER) pathway, which, when lost, alter sensitivity of BRCA2 deficient cells to DNA damaging agents – especially those that cause stalled replication forks. We have identified BER proteins, APE1 (apurinic/apyrimidinic endonuclease) and UNG (Uracil-DNA glycosylase), which, when depleted, rescue sensitivity of BRCA2 deficient cells to stalled fork inducing agents. Depletion of these proteins also reverses chromosomal aberrations, a marker of genomic instability, in BRCA2 depleted cells. Importantly, we find that endogenous base damage is highly elevated in BRCA2 depleted cells. Based on our preliminary data, we hypothesize that altered BER activity and/or APE1 or UNG levels affect cancer risk predisposition in BRCA2 mutation carriers. In keeping with these hypotheses, a 5-year survival analysis of women with BRCA2-mutant ovarian cancer shows that patients with low APE1 levels in their tumor have a far worse prognosis compared to those with high APE1 levels. In order to investigate these hypotheses, we propose to determine whether APE1 and/or UNG affect stalled fork resolution in BRCA2 depleted cells (Aim 1). We will also determine the basis for increased base damage in replicating BRCA2 depleted cells, and the molecular mechanism by which APE1 and UNG affect the resolution of DNA damage in these cells (Aim 2). Finally, we will also determine whether phenotypically normal BRCA2mut/+ mammary epithelial cells (MECs) demonstrate altered stalled fork resolution upon loss of APE1 and/or UNG and how response to therapeutics in BRCA2 mutant cancers is affected by levels of APE1 and/or UNG in those cells (Aim 3). Successful completion of this work will give us an insight into a) a heretofore unknown function of BER proteins in affecting resolution of fork associated DNA damage in BRCA2 depleted cells , b) whether alterations in BER pathway has the ability to influence BRCA2 mutation driven tumorigenesis, and/or the response of BRCA2 tumors to chemotherapeutics, d) source of increased base damage in BRCA2 depleted cells, and c) the mechanism that drives these phenomena. Such work will lay the foundation for targeted, future preventive and therapeutic strategies for BRCA2 mutation associated cancer.

DCB Branch DNA and Chromosome Aberrations

Program Director OKANO, PAUL

Grant 1 R15 CA252990-01A1

PI Name(s) RAPPA, GERMANA; LORICO, AURELIO (contact)

Title Nuclear Transport of Extracellular Vesicle Biomaterials

Institution TOURO UNIVERSITY OF NEVADA

Abstract Targeting the interaction between cancer and stromal cells in the tumor microenvironment holds great therapeutic potential. Recently, interest in this type of communication has expanded beyond signaling molecules to include extracellular vesicles (EVs), released by cancer cells and taken up by target cells locally or at distance. Cancer EVs contain proteins and nucleic acids responsible for pro-tumorigenic and pro-metastatic effects. Our knowledge of the EV world is in its infancy. What happens to EV cargo molecules once inside the target cells, and how they exert their biological effects is still obscure. Our long-term goal is to understand the intracellular route(s) and subcellular fate of EV content upon internalization and to apply this knowledge to developing novel therapeutic strategies, especially in cancer. We have recently identified a novel EV nuclear pathway in which a tripartite VOR protein complex, containing VAP-A, ORP3 and Rab7, orchestrates translocation and docking of EV-containing late endosomes into nuclear envelope invaginations (NEI) with subsequent nuclear transfer of EV cargo. Preliminary evidence of the biological relevance of this novel nuclear pathway was shown by the finding that ORP3 and VAP-A are required for pro-metastatic morphological changes of non-metastatic colon carcinoma cells induced by EVs from metastatic colon carcinoma cells. Our central hypothesis is that this intracellular pathway mediates many of the effects of EVs, and that interfering with mechanism/s that regulate i) the interaction between late endosomes and nuclear membrane, and ii) the nuclear delivery of the endocytosed EV components will impair the intercellular crosstalk in the cancer microenvironment, and thereby inhibit tumor growth and formation of metastases. We propose to investigate the effects of nuclear delivery of colon cancer-derived EV biomaterials on MSCs and to carefully define the proteins involved in this novel nuclear pathway. This objective will be accomplished by two specific aims: (1) we will investigate whether this nuclear pathway is required for the transformation of MSCs by EVs derived from metastatic colon carcinoma cells, paying particular attention to its impact on MSC proliferation, migration and invasiveness; (2) we will dissect in detail the VOR complex by deletion or mutation of domains and/or motifs reportedly involved in protein-protein and protein-lipid interactions, and by expression of VAP-A, ORP3 and/or Rab7 mutants in cells depleted of the corresponding gene. In addition, we will investigate whether other proteins and nuclear pore components are involved. This study is innovative because (a) is based on our discovery of a novel EV nuclear pathway and (b) the nucleus as a final destination of EV cargo has not yet been thoroughly investigated. The proposed project is significant because it will (a) clarify a novel and poorly characterized nuclear pathway, (b) strengthen the research environment of our Institution and involve medical students in all phases of the proposed research, (c) open the way to innovative therapies for cancer metastatic disease targeting the tumor microenvironment.

DCB Branch Tumor Biology and Microenvironment

Program Director WOODHOUSE, ELIZABETH

Grant 1 R15 CA280767-01A1

PI Name(s) RIESE, DAVID J

Title Is ERBB4 a Driver of BRAF WT Melanomas?

Institution AUBURN UNIVERSITY AT AUBURN

Abstract Combining immune checkpoint inhibitors with BRAF and MEK inhibitors has enabled significant improvements in treating metastatic skin cutaneous melanomas that possess activating mutations in the BRAF gene (“BRAF mutant melanomas”). In contrast, actionable and specific targets in metastatic cutaneous melanomas that contain the wild-type BRAF gene (“BRAF WT melanomas”) have yet to be discovered, hindering the development of more effective strategies for treating these tumors. Here we propose to address that knowledge gap by validating a candidate target in BRAF WT melanomas and strategies for therapeutic intervention. Published and preliminary data have led to the following hypothesis: ERBB4 mutations or elevated ERBB4 transcription increase signaling by ERBB4-EGFR or ERBB4- ERBB2 heterodimers, resulting in increased PI3 kinase signaling, cooperation with NF1 or RAS gene mutations, and increased proliferation of BRAF WT melanomas. We will test aspects of this hypothesis with two specific aims: (1) Which ERBB4 mutants found in BRAF WT melanomas cause increased proliferation of BRAF WT melanoma cell lines? (2) Is EGFR, ERBB2, or the PI3K pathway required for proliferation of the ERBB4-dependent, BRAF-WT melanoma cell lines? We estimate that the predicted outcome of these studies will lead to effective strategies for treating approximately 30% of metastatic BRAF WT melanomas, revolutionizing the care of patients that carry these aggressive skin cancers.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant 1 R15 CA267836-01A1

PI Name(s) SABBATINI, MARIA EUGENIA

Title Characterizing the fibrogenic role of NADPH oxidase 1 in the transition from chronic pancreatitis to pancreatic cancer

Institution AUGUSTA UNIVERSITY

Abstract The problem: Patients suffering from chronic pancreatitis (CP) have a higher risk of pancreatic ductal adenocarcinoma (PDAC). CP is characterized by an activated pancreatic stellate cell (PaSC)-rich stroma, which has facilitated the progression of non-invasive PanIN lesions to invasive PDAC. A critical barrier to progress in preventing the CP-to-PDAC transition is the gap of knowledge regarding the mechanism by which quiescent PaSCs become activated by inflammatory mediators, expand, and synthesize stroma and matrix metalloproteinases (MMPs), facilitating the progression of non-invasive PanIN lesions to invasive PDAC. Among the inflammatory mediators of CP are reactive oxygen species (ROS), which activate PaSCs. ROS generation can occur as a primary product of NADPH oxidase (Nox) enzymes. Previously, we showed that Nox1 signaling in CP-activated PaSCs: i) forms fibrotic tissue, ii) up-regulates both the transcription factor E-cadherin repressor Twist1 and MMP-9, and iii) facilitates the invasion of pancreatic cancer cell lines both in vitro and in vivo. The objective: To address the gaps in our knowledge regarding the mechanisms by which Nox1/Twist1/MMP-9 signaling in CP-activated PaSCs facilitates the progression of non-invasive PanIN lesions to invasive PDAC. The central hypothesis: The induction of CP generates ROS by Nox1 in PaSCs, which lead to a sustained expression of Twist1. Twist1, in turn, induces the expression of MMP-9, which promotes the progression of non- invasive PanIN lesions to invasive PDAC by degrading the basal lamina. The hypothesis will be tested by pursuing two specific aims: 1) Under the first aim, we will test in vitro the prediction that inhibiting Nox1/Twist1/MMP-9 signaling in CP-activated PaSCs attenuates the degradation of collagen IV and laminin. Under the second aim, we will test in vivo the prediction that inhibiting Nox1 signaling in CP-activated PaSCs prevents the progression of non-invasive PanIN lesions to invasive PDAC by attenuating the degradation of basal lamina. Our outcomes will include 1) new knowledge of CP-related mechanisms of progression of PDAC, 2) new knowledge of Nox1-related mechanisms of progression of PDAC, 3) high impact research experiences for undergraduate students. The approach is innovate because it will assess the extent to which the generation of Nox1-derived ROS from CP-activated PaSCs can facilitate the progression of non-invasive PanIN lesions to invasive PDAC. The proposed research is significant because a finding that the lack of Nox1 in CP-activated PaSCs prevents the progression of PanIN lesions to invasive PDAC by attenuating the degradation of basal lamina will establish the feasibility and premise for future translational research directed at developing rational, stroma-targeted therapies of early-stages of PDAC (e.g., carcinoma in situ) that, in combination with other approaches might lead to clinical strategies to increase the survival of patients. Moreover, these studies are designed to promote opportunities for a diverse undergraduate student participation providing them with exposure to hands-on experiments and close mentoring.

DCB Branch Tumor Biology and Microenvironment

Program Director WOODHOUSE, ELIZABETH

Grant 1 R15 CA280699-01

PI Name(s) SANABRIA, HUGO

Title Impact of pathogenic missense mutations on the ARID domain of ARID1a

Institution CLEMSON UNIVERSITY

Abstract The AT-rich interacting domain-containing protein 1a (ARID1a), also named BAF250a, p270, or hOSA1, is a vital component of the Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex. ARID1a, as part of the SWI/SNF complex, is responsible for crucial nuclear activities, including regulating transcription, DNA synthesis, DNA damage repair, and cell proliferation. Consequently, ARID1a is classified as a tumor suppressor gene and it is frequently mutated in solid tumor malignancies cancers, amounting to ~6% of every cancer and ~45% of all ovarian cancers. Loss of function mutations in ARID1a due to frameshift, stop-gain, or missense mutations leads to dysregulation of many gene pathways, including the prevention of tumor suppressor activities. Although the loss of function is evident in stop-gain and frameshift mutations, the impact of pathogenic missense mutations is subtler and more difficult to understand or predict. We hypothesize that pathogenic missense mutations impact the protein stability, DNA binding affinity, and structural dynamics, perturbing its function. Specifically, we seek to understand the effects of pathogenic missense mutations in the ARID domain of ARID1a, which is responsible for its direct interaction with DNA. Therefore, the broad goal of this research program is to understand the effects of pathogenic missense mutations in the ARID domain of ARID1a. The long-term implications of this program are the development of a unique framework that will pave the way for directly probing identified pathogenic missense mutations for future guided patient-specific screening therapeutic approaches. Our proposed study integrates biophysical studies, computational approaches, and single-molecule spectroscopy to characterize the stability, DNA binding, and structural dynamics of the ARID domain. Our studies will give insights into the correlation between missense pathogenic mutations and the structure-dynamics-function relationship. The PI and our research team are uniquely positioned to pursue the following specific aims: (1) to determine the impact of pathogenic missense mutations on the stability of the ARID domain; (2) to determine the impact of pathogenic missense mutations in the binding affinity of ARID domain to DNA; and (3) to determine the changes in the structure and dynamics of ARID domain as perturbed by pathogenic missense mutations. This interdisciplinary project will engage undergraduates in research to foster their interest and career development in both physical sciences and cancer research. The expected outcome will be a structural model of ARID1a's ARID domain interaction with DNA, a library of pathogenic mutations ranked by the impact on stability and affinity, a system to improve pathogenic predictors, and the foundation for developing novel therapeutics and personalized medicine in the fight against cancer.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director AMIN, ANOWARUL

Grant 1 R15 CA274480-01A1

PI Name(s) SHI, HAIFEI

Title Estrogenic protection against colorectal cancer development in obesity

Institution MIAMI UNIVERSITY OXFORD

Abstract Sporadic colorectal cancer (CRC), a subtype of CRC without family history but attributed to the presence of various risk factors, is the majority (~75%) of new CRC cases in the US. Obesity and related chronic low-grade inflammation are significant risk factors for sporadic CRC. Although obesity prevalence is higher in women than in men, premenopausal women have lower incidences of CRC than age-matched men. Epidemiology studies have also indicated that postmenopausal women increase their risks for CRC, but women with estrogen replacement therapies have a substantially lower incidence in CRC. These observations suggest proinflammatory adipokines and cytokines associated with obesity as oncogenic factors, whereas estrogen as a protecting factor in CRC development. However, how estrogen suppresses adipokine- and cytokine-induced CRC pathogenesis is unclear. This knowledge gap is mainly because adipokines/cytokines and estrogen have been studied as independent factors in separate studies, but their interaction has not been explored in CRC. Additionally, although cancer cells have impaired mitochondrial metabolic function and elevated anaerobic glycolysis (known as the Warburg effect), most research attention has been focused on studying the underlying cellular and molecular mechanisms, without investigating the metabolic events involved. Further, a suitable obesity-associated CRC animal model is needed to resemble the early histopathologic features leading to sporadic CRC in humans. In this proposal, interaction between estrogen and adipokine leptin or cytokine IL-6 will be studied in in vitro cell and in vivo mouse models to understand how estrogen protects against CRC development in obesity setting, via opposing the oncogenic actions of leptin and IL-6 at cellular, molecular, metabolic, and functional levels. Additionally, estrogen receptor β (ERβ) selective agonist and small interfering RNA transfection to ERβ that specifically reduces ERβ expression will be used to explore estrogenic mechanism. Furthermore, cell lines originally obtained from primary tumors of male and female patients and male and female mice with obesity-promoted colorectal tumorigenesis will be included, considering sex as a biological variable. Findings of this study is invaluable for identifying sex-specific biological targets for intervention to prevent and treat obesity-promoted CRC that would be different between men and women. Importantly, this project provides plentiful opportunities to expose undergraduate students to a broad range of modern techniques such as quantitative real-time PCR, flow cytometry and mass spectrometry, and have hands-on participation in high-quality research using both in vitro and in vivo models. These activities will strengthen research environment in cancer and metabolic research at Miami University, and facilitate collaborative research opportunities involving students of different majors from different colleges at Miami University Ohio.

DCB Branch Tumor Biology and Microenvironment

Program Director MERCER, NATALIA

Grant 1 R15 CA252996-01

PI Name(s) SMALDINO, PHILIP J.

Title Disrupting two cancer hallmarks with one target: DHX36, the major G-quadruplex helicase

Institution BALL STATE UNIVERSITY

Abstract This proposal uses innovative approaches and novel mouse models to define an underlying mechanism of tumorigenesis. This proposal further seeks to identify the major G-quadruplex helicase, DHX36 (aliases: G4R1 and RHAU), as a novel therapeutic target. G-quadruplexes (G4s) are dynamic, “knot-like” DNA or RNA structures that shut down transcription and translation, respectively. Of relevance to cancer, genes that promote cell proliferation and survival (i.e. oncogenes) are more likely to contain G4 sequences, while genes that suppress cell proliferation and survival (i.e. tumor suppressor genes) are depleted of G4s. Therefore, G4s represent an epigenetic feature that generally distinguishes oncogenes from tumor suppresser genes. As such, G4s are attractive cancer therapeutic targets. Moreover, >80% of tumors rely on telomerase to prevent telomere shortening, which confers cellular immortality, a classic hallmark of cancer. Extensive G4 structures form in telomere DNA that inhibit telomerase. Taken together, G4 structures reduce oncogene expression and cellular immortality. Conversely, G4 helicases unwind G4 structures increasing oncogene expression and limiting telomere elongation. DHX36 accounts for the majority of G4 helicase activity in human cells and is commonly overexpressed in cancer. DHX36-overexpression is correlated with a significantly reduction in patient survival. Thus, DHX36 is a prime candidate to explore as a therapeutic target. In this proposal, we will pursue two aims to determine the potential of DHX36 as a therapeutic target. In the first aim, we hypothesize that DHX36- overexpression initiates tumors and exacerbates tumor progression. In the second aim, we hypothesize that decreased DHX36 expression alone or in combination with G4 ligands will reduce oncogene expression, telomere elongation, and result in tumor remission. We will test these hypotheses using a novel Dhx36- overexpression mouse as well as a Dhx36-knockout mouse crossed to a transgenic mouse tumor line. The proposed studies will be the first to determine the role of DHX36 in tumorigenesis using mouse models. This work is poised to identify DHX36 as a novel therapeutic target that inhibition of will disrupt two fundamental cancer pathways: oncogene expression and telomere elongation. Undergraduate and graduate students will be integrated at every stage of the project allowing them to gain authentic experience with innovative mouse and bioinformatics technologies applied to a deadly human disease.

DCB Branch DNA and Chromosome Aberrations

Program Director WITKIN, KEREN L

Grant	1 R15 CA287338-01
PI Name(s)	STAUDINGER, JEFFREY L; CREAMER, BRADLEY ALLEN (contact)
Title	Investigating the Roles of Pregnane X Receptor in Human Breast Cancers
Institution	KANSAS CITY UNIV OF MEDICINE/BIOSCIENCES

Abstract

Breast cancer is the second most common type of cancer diagnosed worldwide and is also the second leading cause of cancer-related deaths among women overall. While much progress has been made in the diagnosis and treatment of breast cancer, traditional chemotherapeutic treatments can lead to the development of chemotherapeutic resistance and remains a major challenge in overall patient outcomes. The activation of the Pregnane X Receptor (PXR) by numerous endo- and xeno-biotics, including several chemotherapeutic drugs, is known to regulate many of the metabolic pathways associated with drug metabolism and resistance. The long-term goal is to understand the transcriptional and metabolic targets of PXR in human breast cancers, and to apply this knowledge in their treatment in order to improve patient outcomes. The objective of this application is to elucidate the effects of PXR expression and activation on the transcriptome and phenotypic changes in human breast cancer cell lines. The central hypothesis, which is formulated on PXRs known roles in drug metabolism in healthy and pathogenic tissues as well as preliminary data, is that PXR activation increases xenobiotic metabolism via the induction of Phase I and II enzymes, as well as Phase III transporters, in addition to alterations in the cell cycle and apoptosis in breast cancer. The rationale for the proposed research is that once the transcriptional and phenotypic alterations in response to PXR activation are identified, pharmacological agents or regimes that interfere with these pathways could be developed to decrease the chemoresistance of breast cancer. The objective of this project will be accomplished by two specific aims: (1) Identify global gene expressions changes in human breast cancers associated with pregnane x receptor expression and/or transcription factor activation. An RNA-seq based approach will be used to identify changes to the transcriptome in response to endogenous and exogenous PXR expression and activation. (2) Examine the effects of PXR expression and activation in models of breast cancer on cell survival and chemoresistance. In- vitro based assays examining the effects of PXR on various aspects of the cell cycle, survival, apoptosis, and invasiveness will be utilized. This study is innovative because it shifts the focus of PXRs roles from normal liver and intestinal drug metabolism in order to fill a large void in our understanding of PXRs influence on breast cancer, and potentially in many other types of cancer as well. The proposed project is significant because it will uncover genes and metabolic pathways that are critical to the development of chemotherapeutic resistance in breast cancer, identifying new potential therapeutic targets.

DCB Branch	Cancer Cell Biology
Program Director	LUO, RUIBAI

Grant 1 R15 CA252987-01

PI Name(s) STECKL, ANDREW JULES

Title Controlled Release of Multiple Drugs from Electrospun Fiber Membranes in the Local Treatment of Glioblastoma

Institution UNIVERSITY OF CINCINNATI

Abstract Glioblastoma multiforme (GBM) is the most aggressive brain tumor with > 90 % recurrence rate and short median survival time (< 15 months). Because GBM recurrence mostly occurs within ~2 cm of the original lesion, a locally diffusing treatment should be very effective to significantly extend median survival. Currently, carmustine (bis-chloroethyl-nitrosourea - BCNU) loaded discs (Gliadel®) provide local drug delivery, implanted into the cavity created after tumor resection. However, there are limitations including a short effective release period (~5-7 days) and non-conformity to the resection cavity due to stiff, unmalleable polymeric disc form. The long-term goal of this proposal is to develop improved methods for controlled, local drug delivery for treating GBM. The objective of this application is to investigate the use of complex, multi-layered fiber membranes as an optimized vehicle for drug delivery. The central hypothesis of this grant, based on successful preliminary animal trial data (with >150-day survival), is that core-sheath fibers formed by coaxial electrospinning can provide a superior drug release profile with controlled initial release and extended long term drug delivery. The core-sheath fiber construct also lends itself to multiple drug release due to its composition of two or more individual components. Multi-layered porous membrane discs and pouches can provide designed (“programmed”) release of drug molecules for “cocktail” therapy. The project consists of three specific aims: (1) Fabricate core-sheath fiber membranes with various polymer hosts for optimum mechanical strength, flexibility, biocompatibility, and ability to incorporate specific drug molecules. Transform planar (thin, large area) membranes into 3-D formulations (discs and pouches) for surgical implantation. (2) Investigate drug release mechanisms from planar membranes, discs and pouches in order to realize programmable long-term (months) drug delivery. Investigate in detail controlled dual drug release from multi-layered core-sheath fiber membrane discs. Sequential drug release was recently demonstrated using TMZ or BCNU and acriflavine (ACF) by embedding ACF-incorporated discs within TMZ or BCNU-incorporated membranes. Combinations of current anti-cancer drugs (BCNU, TMZ, paclitaxel) and potential new drug candidates (ACF, disulfiram) will be investigated to obtain the most synergistic combination for localized cocktail chemotherapy. (3) Demonstrate (a) improved inhibition of cancer cell growth in vitro and (b) extended survival rate using established in vivo animal models. This study proposes the innovative use of complex multi-layered fibers for controlled release of incorporated drug molecules for the treatment of GBM. The project has the potential to provide significant improvement in the outcome of patients with GBM by developing a novel material system for drug delivery in a bioavailable, biocompatible form.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director LI, JERRY

Grant 9 R15 CA254827-02A1

PI Name(s) SUN, HONG

Title Novel Regulation of the Activation and Assembly of the Heterimeric Receptor Tyrosine Kinase Complexes for Cell Signaling

Institution UNIVERSITY OF NEVADA LAS VEGAS

Abstract We propose that AXL, a receptor tyrosine kinase AXL (RTK), can be activated by ligand-independent manner through interaction with another RTK, MET, and form a heterodimeric AXL-MET complex to launch a unique signaling program for cancer cell migration and invasion. MET and AXL are two recently characterized oncogenic RTKs implicated in invasive cell growth and cancer cell migration. Emerging evidence indicates that aberrant activation and overexpression of AXL or gene amplification of MET confer a common resistance mechanism to targeted and conventional therapies in aggressive and metastatic cancers including glioblatoma multiforme (GBM), breast and lung carcinomas. Co-activation of AXL and/or MET with other RTKs such as EGFR or IGF-1R is also recognized as a major hindrance to targeted cancer therapies. The canonical activation of many RTKs involves the binding of a specific ligand to its cognate receptor to promote RTK homo-dimerization to launch a specific signaling cascade. We have recently found that HGF, a natural ligand for MET RTK, induces the activation of a different RTK, AXL, by promoting the formation of MET-AXL hetero-RTK complexes to trigger a novel downstream signaling cascade for cancer cell migration and invasion. Our findings on the formation of MET-AXL hetero-RTK complexes represent a novel and uncharacterized mechanism for activating RTKs. We propose to investigate this novel signaling process that present new targets for future therapies In this application, we will investigate the mechanism by which AXL is activated through interaction with MET to promote cancer cell motility, by conducting the following specific aims: Specific Aim 1. To determine the relationship between homo-RTK and hetero-RTK complexes Specific Aim 2. To identify the novel mechanism underlying the activation and signaling cascade of the MET-AXL hetero-RTK complex. Specific Aim 3. To determine the effects of selective depletion of the p140 form of AXL. As co-activation of RTKs is critically important in GBM and in a multitude of human cancers, elucidation of this new regulatory mechanism may provide novel targets for prevention and therapeutic treatment.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI

Grant	1 R15 CA246335-01A1
PI Name(s)	TILBURY, KARISSA BETH; KHALIL, ANDRE (contact)
Title	Coupling Advanced Computational Analyses for Mammography and SHG Imaging for Early Detection of Breast Cancer Tissue Microenvironment Disruptions Accompanying Tumorigenesis
Institution	UNIVERSITY OF MAINE ORONO
Abstract	The breast tumor microenvironment plays a key role in the early development of cancer. An improved understanding of the tumor microenvironment will support the early detection of disease and the development of novel, more effective therapies. Current radiological interpretation of mammograms is based on identifying breast lesions and assessing their potential malignancy and lethality. The biomedical research community has yet to correlate the mammographic signatures of microenvironment alterations and loss of tissue homeostasis accompanying, or even preceding tumorigenesis. The PI recently used a novel and powerful computational technique to demonstrate that tissue disruption and loss of homeostasis can be quantitatively and objectively assessed from standard clinical mammography. These preliminary findings pertain only to single diagnostic mammograms; the proposed AREA project will analyze longitudinal sequences of mammograms over multiple years prior to, and approaching the year of diagnosis, thus revealing the progression of healthy breast tissue microenvironment towards a disrupted state. It is hypothesized that the tissue disorganization signature present in mammographic data is associated with tissue disruption and loss of tissue homeostasis in the microenvironment. Team members will verify this hypothesis by imaging breast tissue samples to characterize, at the cellular level, disruption of collagen structures associated to tumor microenvironment alterations accompanying tumorigenesis. This project's specific aims include: 1) verifying that mammographic tissue disruption accompanies and perhaps even precedes tumor development; 2) developing an in silico model of microcalcification growth into mammographic breast tissue environment, 3) improving the computational efficiency of our data analysis, and 4) linking morphological features of the breast tumor microenvironment imaged by mammography to cellular-scale alterations imaged by second harmonic generation microscopy. The proposed research will advance the education and career preparation of undergraduates within Maine's only biomedical engineering program, nurture a growing partnership among the University of Maine, Spectrum Healthcare Partners and Maine Medical Research Institute, introduce two UMaine faculty to biomedical research, and support the early career development of two assistant professors.
DCB Branch	Biophysics, Bioengineering, and Computational Sciences
Program Director	MILLER, DAVID J

Grant 1 R15 CA263784-01

PI Name(s) TOMIDA, JUNYA

Title Synergistic DNA repair genes and sensitivity to radiation therapy in prostate cancer

Institution UNIVERSITY OF NORTH CAROLINA CHARLOTTE

Abstract

In the U.S, over 33,000 patients die yearly from prostate cancer (PCa), and an estimated 190,000 new cases are diagnosed. 10-20% of these patients are diagnosed with metastatic PCa (mPCa). mPCa and metastatic castration-resistant prostate cancer (mCRPC) acquire resistance to established treatments and progress with profound effects on patient quality of life. mCRPC remains fatal. Our group was the first to describe the new DNA repair gene FAM35A whose status may greatly affect tumor sensitivity to radiation treatment in a large proportion of PCa patients. This R15 project will determine a new mechanism of FAM35A-related treatment resistance/sensitization in PCa. Our central hypothesis is that 1) FAM35A regulates resection inhibition and 2) FAM35A and DNA polymerase theta (POLQ) antagonize the homologous recombination (HR) pathway. We will investigate through the following two specific aims: AIM 1: Determine the mechanism of FAM35A-mediated resection inhibition. Although our FAM35A-complex data includes BLM as a binding partner, it remains unclear how FAM35A blocks activity of resection enzymes (BLM-DNA2 and BLM-EXO1) and/or prevents the interaction of BLM with DNA2 or EXO1. We will test our working hypothesis that FAM35A prevents nuclease activity by binding to DNA and/or by binding to BLM. Our experimental approach will incorporate biochemical and cell biology methods to determine FAM35A's resection inhibition activity. First, we will a) confirm in vitro DNA-binding activity of the two FAM35A isoforms, and b) determine the BLM binding site in FAM35A. Using FAM35A knockout cell lines complemented with mutant FAM35A, we will then assess DNA damage sensitivity using clonogenic assays; monitor the DNA damage response using confocal microscopy; and measure resection using DNA fiber assays. AIM 2: Elucidate the mechanism by which FAM35A contributes to the DNA damage response in POLQ KO PCa. My working hypothesis is that depletion of FAM35A/POLQ causes complete dysfunction of both c- and alt- NHEJ pathways, resulting in HR hyperactivation. 1) To test how FAM35A deficiency affects DNA damaging agents with and without POLQ, we will perform clonogenic assays using three FAM35A-depleted PCa cell lines of varying POLQ status. 2) To determine DNA repair activity in FAM35A-depleted POLQ KO PCa cells treated with DNA damaging agents, we will use confocal microscopy and immunoblotting to monitor the DNA damage response with DNA damage inducing agents. We will perform DSB-induced HR assays to measure HR activity. Results are expected to clarify the role of DNA repair dysfunction in resistance/sensitization in PCa and facilitate discovery of therapeutic and prognostic targets for radiation. The primary positive impact will be clarification of roles of DNA repair pathway between FAM35A and Polq. This AREA project will enable the research training of undergraduate and graduate students pursuing careers in health and basic sciences.

DCB Branch DNA and Chromosome Aberrations

Program Director WITKIN, KEREN L

Grant 1 R15 CA267864-01A1

PI Name(s) VELEZ-CRUZ, RENIER

Title The role of SWI/SNF chromatin remodelers in homologous recombination and genome stability

Institution MIDWESTERN UNIVERSITY

Abstract Multiple subunits of the SWI/SNF chromatin remodeling complexes have been identified as a novel class of tumor suppressors mutated in up to 20% of human cancers, but their mechanism of tumor suppression is still poorly understood. These complexes are composed of a mutually exclusive ATPase subunit (either BRG1 or BRM), a set of core factors, and a group of accessory subunits. SWI/SNF complexes were first identified as transcriptional activators and therefore most of the research efforts have been focused on the role of these ATPases in the transcriptional regulation of a variety of cellular pathways involved in carcinogenesis. More recent work has linked BRG1 to DNA repair. We identified a complex containing TopBP1-E2F1-RB responsible for the recruitment of BRG1 to DNA double strand breaks (DSBs). Moreover, we also identified a function for BRG1 stimulating DNA end resection and homologous recombination (HR) by reducing nucleosome density at DSBs and promoting the recruitment of the CtIP nuclease. However, the role that the other ATPase (BRM) and other non-catalytic subunits of the SWI/SNF complex may play in this function is still unknown. The goal of this proposal is to further define the mechanism by which BRG1 and BRM stimulate DNA end resection and HR, to determine to what extent SWI/SNF subunits ARID1A and BRD7 are required for this function, and to test whether we can sensitize breast cancer cells to chemotherapeutic agents by inactivating these SWI/SNF subunits. Our hypothesis is that BRG1 reduces nucleosome density at DSBs, thus allowing the recruitment of the DNA end resection nuclease CtIP and stimulating DNA end resection and HR, and that ARID1A and BRD7 are important for anchoring these complexes at DSBs. The uncovering of a function in DSB repair for SWI/SNF accessory subunits could help explain their tumor suppressor activities. Moreover, identifying multiple SWI/SNF subunits required for HR, would also identify a DNA repair vulnerability that could be exploited therapeutically against SWI/SNF-mutated cancers. In order to test this hypothesis, we will, first, determine whether a BRG1- or BRM- ATPase-dead mutant would be able to stimulate DNA end resection and promote the recruitment of the CtIP nuclease. Second, we will define the role non-catalytic SWI/SNF subunits ARID1A and BRD7 play in the recruitment of BRG1, chromatin remodeling at DSBs, DNA end resection, and HR, by analyzing the generation of single-stranded DNA and ATR signaling after DNA damage. Third, we will determine whether we can sensitize breast cancer cells to chemotherapeutic agents, including Poly (ADP-ribose) polymerase (PARP) inhibitors, by inactivating BRG1, BRM, ARID1A, and BRD7 subunits. This systematic study of the function of the SWI/SNF subunits in DNA end resection and HR will further our understanding of the biology of these complexes, their functions, and the required composition of these complexes in order to perform this function in HR. Moreover, we could potentially identify DNA repair vulnerabilities in SWI/SNF-mutated cancers that could be exploited through specific chemotherapeutic regimens designed for HR-deficient malignancies.

DCB Branch DNA and Chromosome Aberrations

Program Director WEINREICH, MICHAEL DALE

Grant 1 R15 CA254006-01A1

PI Name(s) VILLA DIAZ, LUIS GERARDO; MADLAMBAYAN, GERARD JAMES (contact)

Title The Role of Hematopoietic Stem and Progenitor Cells in Solid Tumor Growth and Response to Radiation Therapy

Institution OAKLAND UNIVERSITY

Abstract A major component of many solid tumors, including lung cancers, are bone marrow (BM)-derived immune cells that migrate to tumors and aid in their continued growth. While the activity of these cells including tumor associated macrophages (TAMs) has been the subject of intense investigation, we recently identified that BM- derived hematopoietic stem and progenitor cells (HSPCs) are also present in growing tumors and can be functionally maintained intratumorally for long periods of time. Interestingly, the numbers of HSPCs present in tumors directly correlates to the eventual regrowth rates of tumors following radiation therapy (RT). The data suggests that HSPCs represent another important cell population involved in tumor biology, however; their mechanism of action is still unclear. Filling this gap in knowledge will add to the ever-changing understanding of tumor biology. The objective of this proposal is to determine how HSPCs are maintained in tumors and how HSPCs promote tumor regrowth post-RT. Our preliminary data support the idea that HSPCs are maintained through interactions of the integrin CD49f and laminins present within the tumor extracellular matrix. In Specific Aim 1, we will show that this interaction is indeed responsible for HSPC maintenance using in vitro and in vivo strategies that block or enhance this interaction followed by analysis of their effects on HSPC functionality. We will also define the intracellular signaling pathways involved in this process with initial studies focusing on focal adhesion kinase (FAK) signaling. These studies will characterize for the first time a tumor specific niche capable of maintaining HSPCs outside of the BM. In Specific Aim 2, we will demonstrate that tumor treatment with RT exacerbates HSPC migration to tumors and concomitantly disrupts the interaction between CD49f and laminin. We will also show that RT produces tumor microenvironments that favor the differentiation of these ‘released’ HSPCs into tumor supportive macrophages (specifically M2 polarized) to aid in tumor recovery. We will also test the effects of blocking the activity of HSPCs on tumor growth and regrowth post-RT. By completing the proposed studies, our long-term goal is to use the knowledge gained to make a significant contribution towards the development of more robust treatment strategies for patients suffering with solid tumor based cancers.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant 1 R15 CA242057-01A1

PI Name(s) WALLACE, NICHOLAS A

Title HPV Oncogenes Dysregulate Translesion Synthesis

Institution KANSAS STATE UNIVERSITY

Abstract Cervical cancers (CaCxs) cause over 250,000 deaths annually are the result of human papillomavirus (HPV) infections. The combination of low HPV vaccine uptake and the average time required for an HPV infection (10-30 years) to cause a CaCx, mean that improving treatment options will remain essential for the foreseeable future. CaCxs require continued HPV oncogene (HPV E6 and E7) expression for initiation and maintenance making these viral oncogenes an attractive target for intervention. Because direct attacking HPV E6 and E7 remains technically difficult, we propose targeting their manipulation of cellular signaling. We identify translesion synthesis (TLS) dysregulation in cervical cancers (CaCx) and show that HPV E6 and E7 cause this aberrant signaling. TLS helps cells tolerate replication stress by preventing replication forks from stalling and collapsing. It exchanges the high fidelity replicative polymerase for a TLS-polymerase (often POLη) that can synthesize DNA with lower fidelity in adverse conditions. HPV E7 causes replication stress by allowing bypass of G1/S-phase checkpoints and depleting nucleoside reserves. This activates TLS, but HPV E6 prevents completion of the pathway by blocking POLη accumulation. We found both HPV oncogenes were capable of preventing POLη induction in response to DNA damaging UV exposure. Both of these phenotypes correlate with increased genome instability and sensitivity to exogenous replication stress (mitomycin C, cisplatin and UV). Our overall hypothesis is that by taxing and inhibiting TLS HPV oncogenes impair genome fidelity in cervical cancers. We test this hypothesis in two related, but distinct specific aims. Aim 1 investigates the destabilizing created by HPV oncogene's simultaneous activation and inhibition of TLS using primary keratinocytes (the cell type HPV infects) that acutely or constitutively express HPV oncogenes. The mutagenic consequences are defined using whole genome sequencing and established molecular approaches, like immunofluorescence microscopy and immunoblotting. Aim 2 determines the mechanism and consequences of the aberrant TLS response to cisplatin in CaCx. This aim combines specialized approaches (e.g. visualization of metaphase chromosomes) and other molecular techniques. It characterizes TLS in cells after UV and cisplatin exposure, defines the mechanism by which HPV E6 and E7 prevent POLη induction in these cells, and examines the consequences of this TLS hindrance. Undergraduate Student Involvement. Students are the bedrock of this proposal. They were instrumental in generating the corroborative data and will continue to be integral in the proposed experiments. We anticipate preparing 5 undergraduates for futures in research.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant	1 R15 CA274349-01
PI Name(s)	WANG, CONGZHOU
Title	Reversing epithelial-mesenchymal transition in metastatic cancer cells using engineered nanomaterials and a mild photothermal effect
Institution	SOUTH DAKOTA SCHOOL OF MINES AND TECHN'Y

Abstract

Tumor metastasis accounts for 90% of cancer-associated mortality. Epithelial-mesenchymal transition (EMT) of cancer cells provides the cancer cells with strong migratory-invasive abilities, and more recently, to mediate the development of chemoresistance, which is linked to cancer stem cell-like features. There is growing evidence suggesting that targeting EMT can be used as a therapeutic approach itself, or to enhance the efficacy of other anticancer treatments. However, the translation of EMT targeted compounds to the clinic has been challenging due to the lack of molecular-cellular targeting specificity and efficacy. Moreover, most of these compounds only focus on the prevention of EMT, but not on the tumor cells that have already undergone EMT. Innovations that are more specific, effective, and broadly applicable to eliminate EMT-type cancer cells with high metastatic capability and enhanced drug resistance hold great potential to revolutionize the treatment of tumor metastasis. In this project, we will develop a biomaterial-based approach to reverse the EMT of cancer cells by targeting a transmembrane EMT inducer, CD146, using engineered black phosphorus nanosheets (BPNs) and a mild photothermal effect. Our central hypothesis is that CD146 targeted BPNs and a mild hyperthermia will synergistically reverse the EMT in cancer cells, and thus stop the cancer cell migration and sensitize the cancer cells to classical chemotherapy drugs. Our preliminary studies have been able to demonstrate this approach in reversing the EMT of breast cancer cells, leading to nearly stopped cancer cell migration. As CD146 is overexpressed on a series of metastatic cancer cells, we will extend this approach to two other cancer types (including prostate cancer and melanoma), and uncover its working mechanism, which will lay the foundation for the next phase in vivo studies. Three aims have been set to test the hypothesis. Aim 1 will synthesize, optimize, and characterize CD146 targeted BPNs, and evaluate the approach based on CD146 targeted BPNs and a mild hyperthermia for the inhibition of cancer cell migration. Aim 2 will evaluate the efficacy of this approach in reversing the EMT process in cancer cells and the effectiveness of this approach in sensitizing the cancer cells to classical chemotherapeutics. Aim 3 will elucidate the molecule mechanism of how this approach reverses the EMT process in cancer cells. The proposed research is transformative in that (i) it will offer a new perspective for treating cancers by eliminating EMT-type cancer cells and minimizing their invasiveness and chemoresistance; and (ii) the mechanistic knowledge generated by this work will inform the future design of biophotonic nanomaterials to modulate cell phenotypes for in vivo cancer treatment and beyond. This research will also strengthen undergraduate research activities in biomedical engineering at the South Dakota School of Mines and Technology by recruiting nine undergraduate students into the proposed research (three for each year). Students will gain substantial knowledge and skills in biomedical research, which will prepare the students for a successful career in biomedical and health sciences.

DCB Branch	Tumor Metastasis
Program Director	GRIL, BRUNILDE M

Grant 1 R15 CA252986-01A1

PI Name(s) WANG, LING

Title The Ubiquitin Sensor p62 Is A Novel Component of EBV LMP1 Signalosome

Institution EAST TENNESSEE STATE UNIVERSITY

Abstract Epstein-Barr Virus (EBV), the first identified human cancer virus, is associated with a panel of malignancies of lymphocytic and epithelial origin, and serves as a paradigm for the study of host-virus interaction. EBV is well known to manipulate the host ubiquitin machinery to facilitate its latent persistence and oncogenesis, examplified by EBV LMP1 signal transduction to the activation of multiple transcription factors, such as NFκB and those we have identified including IRF7/IRF4, which control immune response and inflammation, as well as cell survival and growth. Constitutive and well balanced activation of LMP1 signaling is crucial for survival of EBV-transformed cells, and its depletion or overexpression leads to cell death. It is therefore vital to delineate the detailed mechanisms underlying LMP1 signal transduction for understanding EBV-mediated oncogenesis. p62 (also called SQSTM1, Sequestosome 1) is a ubiqutin sensor and a signal transducing adaptor that interacts with TRAF6 and facilitates the recruitment of ubiquitinated signal intermediators for the activation of NFκB in diverse contexts. In turn, p62 is induced by NFκB activity. EBV LMP1 is known to activate NFκB in its latency. However, the interaction between p62 and EBV latency has never been studied. We have recently published interesting and important results, which imply p62 in LMP1-mediated functions in EBV latency. We further show that p62 is upregulated in EBV latency 3, depending on LMP1/NFκB pathway activity, and that p62 interacts with LMP1 and shRNA-mediated p62 depletion in LCLs reduces cell proliferation. Thus, we hypothsize that EBV latent infection induces p62 expression through LMP1 signaling, and in turn, p62 participates in LMP1 signal transduction leading to NFκB activation. We propose to study: Aim 1. The transcriptional regulation of p62 by the LMP1/NFκB and LMP1/AP1 pathway axes; Aim 2. The role of p62 in LMP1 signaling to NFκB activation in EBV latency, including the underlying mechanisms, which include: a) p62-TRAF6 interaction; and b) p62 as a ubiquitin sensor that facilitates the recruitment of signal molecules. Findings from this study will identify p62 as a novel and critical player in EBV LMP1 signaling, and long-term pursuits may identify p62-mediated functions as a potential therapeutic target for EBV-associated malignancies. This proposal involves a series of techniques spanning different biomedical disciplines, which provide an excellent training opportunity for students to establish their interests in biomedical research by being involved in experimental design, critical scientific thinking, problem solving, and scientific writing and presentation.

DCB Branch Cancer Immunology, Hematology, and Etiology

Program Director DUGLAS TABOR, YVONNE

Grant	1 R15 CA263868-01
PI Name(s)	WEISSMILLER, APRIL M
Title	Determining the significance of the N-MYC-WDR5 interaction in neuroblastoma.
Institution	MIDDLE TENNESSEE STATE UNIVERSITY

Abstract

Neuroblastoma (NB) is the most common extracranial solid tumor found in infants, with almost all cases being diagnosed by the age of five. The most severe cases of neuroblastoma are linked to amplification of the N-MYC oncogene, which occurs in ~20% of all NB. N-MYC amplification is associated with ~50% overall survival despite aggressive multimodal therapies, highlighting the need for new targeted therapies to combat this pediatric cancer. N-MYC, like any MYC protein, must be able to recognize and bind chromatin in order to activate its oncogenic potential. This project is built on the premise that an essential co-factor for MYC proteins called WDR5 acts to recruit N-MYC to chromatin at genes that work to maintain neuroblastoma function, and that targeting the N-MYC-WDR5 interaction can serve as a focal point for anti- N-MYC based therapies. Support for this hypothesis comes from published data showing that WDR5 is a conserved regulator of protein synthesis genes across multiple cellular contexts where WDR5 binds and regulates these loci through its so-called “WIN”-site. In addition, the MYC family member, c-MYC, requires WDR5 to bind genes associated with biomass accumulation and translation, and the c-MYC-WDR5 interaction is essential for tumor maintenance in lymphoma mouse models. In an N-MYC amplified NB cell line, preliminary data reveal that targeting the WIN-site of WDR5 using small molecule inhibitors results in a genome-wide decrease in WDR5 binding, with a subsequent loss of N-MYC binding at specific N-MYC- WDR5 co-bound genes. And, importantly, disrupting the N-MYC-WDR5 interaction using genetic mutants impairs the ability of N-MYC to drive anchorage-independent growth, providing evidence that there are essential tumor functions tied to N-MYC that require interaction with WDR5. Together these data provide a solid foundation for the notion that N-MYC requires WDR5 to bind and regulate N-MYC-WDR5 co-bound genes that are important for driving N-MYC specific activities. The goal of this project is to interrogate the N- MYC-WDR5 interaction in N-MYC amplified neuroblastoma cell lines and determine the influence that WDR5 has on N-MYC driven transcriptional processes and neuroblastoma function using genetic and chemical perturbations. Specific Aim 1 will employ genetic and genomic approaches to identify the genes that are bound by N-MYC and WDR5, and determine at which genes the recruitment of N-MYC is dependent on the N-MYC-WDR5 interaction. Specific Aim 2 will combine high-resolution transcriptomic analyses with cellular and in vivo functional assays to challenge the significance of the N-MYC-WDR5 interaction. At the completion of these studies we will have identified the precise genes in which N-MYC binding and transcription is regulated by the N-MYC-WDR5 interaction, and have directly challenged the significance of the N-MYC-WDR5 interactions on multiple facets of NB tumorigenesis.

DCB Branch	DNA and Chromosome Aberrations
Program Director	FINGERMAN, IAN M

Grant 1 R15 CA253134-01

PI Name(s) YATSUNYK, LILIYA A

Title Deciphering the structure and dynamics of non-canonical DNA implicated in cancer

Institution SWARTHMORE COLLEGE

Abstract The proposed research will improve the selectivity and efficacy of anticancer therapies by contributing new knowledge about non-canonical nucleic acid structures, G-quadruplexes (GQ) and i-motifs, and details of their interactions with small-molecule ligands. Bioinformatics studies have identified 700,000 sequences with GQ- forming potential in the human genome. The C-rich opposite strands are proposed to form i-motifs. There is now convincing biological evidence that GQs and i-motifs form in vivo and that these structures complement each other in regulating a variety of cancer-related biological processes. GQ nucleic acids have been firmly established as an important therapeutic target for cancer. The same evidence for i-motifs is steadily accumulating. Small molecules that bind selectively to GQ DNA and RNA and to i-motifs have been identified, and some have been shown to inhibit tumor cells growth; however, exact mechanisms underlying this inhibition are not known. Additionally, the number of selective i-motif ligands is low. Such ligands may ultimately become lead compounds for cancer intervention superior to conventional mutagenetic therapies. Nucleic acid-centered drug discovery programs suffer from limited structural information for GQs and i-motifs, especially in the presence of ligands. As of now, no structure of an i-motif-ligand complex has been reported. The situation is further complicated by high structural diversity of both GQs and i-motifs, their contradictory biological functions, and our limited ability to target their specific folding topology (e.g., parallel vs antiparallel GQs). To address these challenges, we propose to perform comprehensive crystallographic investigation of telomeric and oncogene promoter GQs and i-motifs, both alone and in complex with novel and commercially available selective small-molecule ligands. The diversity of interactions which provide stability to GQs and i-motifs will be determined. The details of ligand binding sites, as well as chemical and structural features of ligands essential for their affinity and selectivity will be identified. This work will be complemented by spectroscopic and calorimetric studies of the thermodynamic parameters of ligand binding. For GQ DNA, that is much more explored, structural studies will be complemented by rigorous kinetic exploration of ligand-assisted GQ folding. Kinetic information can help us identify the timescale of GQ formation and, thus, biological processes that can be affected by the presence of these structures. Collectively, the proposed work will enhance our understanding of GQ and i-motif structural plasticity, supply coordinates for drug discovery platforms, shed light on the origin of ligand selectivity for a specific DNA or RNA target, and guide the design of novel anticancer therapies all while providing transformative training to Swarthmore undergraduates.

DCB Branch Biophysics, Bioengineering, and Computational Sciences

Program Director AMIN, ANOWARUL

Grant 1 R15 CA287406-01

PI Name(s) ZHU, YAN

Title ELUCIDATING THE FUNCTION OF P53-MEDIATED IL17RB REPRESSION

Institution ST. JOHN'S UNIVERSITY

Abstract In addition to its pivotal roles in regulating cell cycle arrest, apoptosis, DNA repair, and metabolism, the p53 tumor suppressor has been demonstrated to modulate innate immune and adaptive immune responses through its crosstalk with key regulators of immune signaling pathways. The dysregulation of Interleukin 17 (IL17) cytokine family and its receptors has been associated with many human diseases, notably inflammation and cancer, crediting to their crucial roles in normal host immune responses. Particularly, Interleukin 17 Receptor B (IL17RB) has been found to be overexpressed in various cancers through the activation of NF B, AKT, or ERK signaling to promote tumorigenesis. Our preliminary results indicate that IL17RB is a p53 repression target and repression of IL17RB sustains the p53 response. Additionally, activation of p53 by nutlin-3a or IL17RB depletion decreases the expression of pro-inflammatory cytokine IL8 induced by lipopolysaccharide (LPS). The goal of this application is to study the role of p53-mediated IL17RB repression in tumor suppression and inflammation inhibition. We established systems of IL17RB repression, overexpression, and activation, that will enable biochemical and cellular characterization as well as in-depth gene expression analyses to elucidate the contribution of IL17RB repression to tumor suppressive and inflammation inhibitory function of p53. The proposed work will not only allow us to gain a better understanding on how IL17RB links p53's crosstalk with other signaling pathways and how p53 attenuates oncogenic effects of chronic inflammation through repressing IL17RB, but also provide a mechanistic basis for the development of novel anti-cancer strategies as well as inflammation inhibitors. In addition, this project will enhance the research environment at St. John's University by providing undergraduate and graduate students with numerous opportunities to learn the fundamentals of biomedical research.

DCB Branch Cancer Cell Biology

Program Director LUO, RUIBAI