

Cancer Research Coordinating Committee

Abstracts for 2020-2021 Awards

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Effects of tobacco and cannabis policy implementation on consumption

Host Campus: San Francisco

Lead Investigator: Dorothy Apollonio

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$51,454

Abstract:

Between 2016 and 2018, California changed multiple policies addressing substance use by legalizing recreational use of cannabis, increasing the minimum age of legal access for tobacco to 21 years, regulating electronic nicotine delivery systems as tobacco products, and expanding access to treatment. The extent to which cannabis policies would be implemented was left to local governments, and varies significantly within the state, with some counties banning retail sales while others allow broad commercialization. As a result, the public health impacts of these changes have been difficult to assess. In particular, stricter tobacco control policies intended to reduce cancer risk may be affected by increased access to cannabis, leading to increased co-use, tobacco-to-cannabis substitution, or both at once in different subpopulations. In this pilot study, we propose to classify tobacco and cannabis policies in California counties and identify associated consumption patterns. We hypothesize that local policies can be classified by the degree to which they restrict access and subsidize treatment, and that policies that do so are associated with reduced consumption of both tobacco and cannabis. We propose the following aims: 1) Map local tobacco and cannabis policies in California and classify their comprehensiveness; and 2) Determine the association between local policies and tobacco and cannabis consumption patterns. We will collect policy data for California counties using data collected by the American Nonsmokers Rights Foundation (ANRF; tobacco) and the Orange County Register (cannabis). We will classify local policies by the degree to which they restrict access to and subsidize treatment for tobacco and cannabis, including treatment policies for co-use. Using data from the 2018 California Health Interview Survey (CHIS), we will analyze the association between local policies and health outcomes: 1) past-month tobacco and cannabis use; 2) modes of use (e.g. smoking, vaping, edibles, dissolvables); and 3) co-use of tobacco and cannabis, (both single-occasion and concurrent). California's choice to devolve implementation of cannabis policies to local governments makes it possible to determine how substance use policies interact to change consumption, and will inform governments within and outside California seeking policy interventions to prevent cancer.

Leveraging the Human Gut Microbiome to Predict Efficacy of Tamoxifen in Breast Cancer Treatment

Host Campus: Irvine

Lead Investigator: Elizabeth Bess

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Despite tamoxifen being one of the most commonly prescribed drugs used to prevent cancer from recurring in breast cancer patients, only 50% of patients are effectively treated with this drug. Accurate prediction of which patients will receive the beneficial effects of tamoxifen is not currently possible. The key to unlocking this mystery may be found in the human gut microbiome, which is composed of trillions of bacteria that reside in the gastrointestinal tract.

Tamoxifen, in addition to being a powerful anti-cancer drug, is also a potent antibiotic that can kill beneficial bacteria in the gut microbiome. Breast cancer patients are treated with tamoxifen for 5–10 years, so injury to the gut microbiome may be severe and long-lasting. As the composition of bacteria in the gut microbiome is linked to numerous diseases, ranging from Parkinson’s disease to breast cancer, clarifying the impact of tamoxifen on the gut microbiome is critical for understanding this treatment’s impact on the overall health of patients. Towards this end, we will perform the first investigation of how communities of gut bacteria may be changed by tamoxifen.

When bacteria are exposed to antibiotics, a common means of avoiding death is breaking down the drug. In the case of tamoxifen, this would likely prevent the drug from being effective at treating breast cancer. If a breast cancer patient were to harbor tamoxifen-degrading gut bacteria, tamoxifen may not treat their cancer. Because gut microbiome composition differs significantly between people, variable presence of tamoxifen-degrading bacteria in patients’ gut microbiomes may explain why some patients do not receive the beneficial effects of tamoxifen. We will perform the first in-depth investigation to identify how gut bacteria resist being killed by tamoxifen, whether tamoxifen is inactivated in this process, and what chemical features of the drug lead to its antibiotic effects.

The research we propose is significant because it is expected to show that the gut microbiome is a missing key to accurately predicting which patients will receive the beneficial effects of tamoxifen. Ultimately, the results of this research may provide new opportunities to achieve personalized and more effective treatment of breast cancer by accounting for the uniqueness of each patient’s gut microbiome.

A DNA Damage Dependent Regulation of APOBEC3A by the Innate Immune System in Cancer Cells

Host Campus: Irvine

Lead Investigator: Remi Buisson

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$83,000

Abstract:

A critical issue in cancer research is understanding tumor heterogeneity, a major cause of resistance to chemotherapy drugs as well as metastasis development. Overcoming resistance and preventing metastasis are the biggest challenges that must be solved to successfully fight cancers and extend patients' lives. Within the same tumor from the same patient, individual tumor cells can dramatically differ from each other. Heterogeneity greatly complicates clinical treatment as some of the cancer cells respond to therapy while others do not. Therefore, there is a critical need to define mechanisms that drive tumor heterogeneity in order to develop new strategies to attenuate tumor evolution and adaptation.

Recently, APOBEC3A (A3A) has been identified as a major driver of tumor heterogeneity. While the importance of A3A in cancer evolution has been clearly established, A3A regulation in cancer cells remains poorly understood which has prevented mechanistic insight in tumor heterogeneity as well as the development of therapeutic approaches that counteract drug resistance. A3A directly attacks genomic DNA causing the deamination of cytosine to uracil and inducing mutations. With its unique ability to rewrite genomic information, A3A is beneficial for tumors because it increases diversity in the tumor, promotes disease progression, and increases resistance to various therapies. Consequently, in patients, A3A mutations are associated with poor prognosis and low survival rate. Ironically, A3A is rarely expressed in tumor patient samples, whereas mutations caused by A3A are commonly found in many types of cancer. From this paradox, we hypothesize that A3A is tightly regulated at the transcriptional level. We propose to identify the initial stress responsible for the up-regulation of A3A expression in tumors and to characterize the molecular mechanism that regulates A3A expression. Our goal is to uncover the molecular mechanisms that govern A3A regulation. Our long-term goal is to develop therapeutic strategies to suppress mutations in the genome caused by A3A leading to tumor heterogeneity.

Control of Cell Fate Commitment by the Histone Chaperone CAF-1

Host Campus: Riverside

Lead Investigator: Sihem Cheloufi

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

During normal homeostasis mature cells are sustained by their stem and progenitor cells. This process involves a delicate balance between proliferation and differentiation when cells become progressively committed. Cancer arises when this balance is perturbed resulting in a deviation from the normal differentiation and uncontrolled proliferation. How cell fate commitment is restricted during normal homeostasis is largely unknown. Understanding the mechanisms that control this process will shed light on how cell identity is maintained and how it is reprogrammed in cancer. We have previously uncovered a role for the chromatin assembly factor complex CAF-1 in safeguarding cell identity during cellular reprogramming. Mechanistically, we discovered that the loss of CAF-1 results in increasing enhancer elements accessibility to the ectopically expressed transcription factors and therefore facilitating cell fate change. However, how CAF-1 affects cell fate commitment during normal homeostasis is poorly understood. Here we propose to investigate the role of CAF-1 during granulocyte macrophage progenitors (GMPs) differentiation. GMPs must replenish billions of terminally-differentiated neutrophils daily under homeostatic conditions and thus presents an ideal platform to study the mechanisms that restrict cell fate commitment. Our preliminary results demonstrate that acute CAF-1 suppression in GMPs triggers their rapid growth arrest and differentiation into neutrophils. Unexpectedly, single cell RNA-seq analysis of CAF-1 depleted GMPs revealed concomitant activation of megakaryocytes and erythroid transcriptional signatures resulting in a mixed unstable cellular state. How CAF-1 normally restricts GMPs differentiation into neutrophils is unknown. Here we propose to dissect the mechanisms by which CAF-1 restricts lineage choice and prevent alternate cell fate change by (1) Characterize chromatin accessibility upon CAF-1 loss to functionally dissect the role of key transcription factors that instruct alternate cell fate change during myeloid differentiation. (2) Interrogate whether the resulting unstable cell state can be reprogrammed to commit down a specific differentiation path by manipulating signaling and transcriptional pathways. These studies will shed light on how CAF-1 control the activity of lineage specific transcription factors and signaling molecules.

Advancing VCP inhibitors as experimental therapeutics in ovarian cancer

Host Campus: Davis

Lead Investigator: Jeremy Chien

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Protein quality control (PQC) pathways are important for protein and organelle homeostasis and essential for the fitness of cancer cells in the face of genomic instability, thus creating a cancer cell dependency. Components of PQC, such as heat shock proteins, also provide an adaptive response to cancer therapy and contribute to resistance. Therefore, PQC represents a point of vulnerability for cancer cells and a therapeutic target to potentiate the efficacy of current cancer therapies. Underscoring this point is a series of reports indicating that various components of PQC are identified as synthetic lethal targets in cancer cells. Among them, valosin-containing protein (VCP, also known as p97 AAA-ATPase) was identified as a lineage-specific dependency gene in ovarian cancer. VCP participates in various cellular functions of protein and organelle homeostasis, and we have shown that VCP inhibition induces ER stress, the terminal unfolded protein response and cytotoxicity in ovarian cancer cells. A previous clinical lead, CB-5083 (CB), inhibits VCP activity in nanomolar concentrations and produces cytotoxicity in over 300 cancer cell lines at low micromolar concentrations. Despite promising in vitro and in vivo anti-cancer activities, a recent first-in-human Phase I clinical trial with CB was stopped due to off-target effects on PDE6, a protein critical for phototransduction, which manifested as ocular side effects. We propose to circumvent the off-target issues using two approaches: (1) nanoformulation of CB to optimize delivery to tumor tissues and (2) the development of CB derivatives as VCP degraders. In the first approach, we will use hydrophobic porphyrin that forms the drug-loadable core with PEG forming the lipid-soluble micelle. Pegylated cross-linked nanoparticles (NPs) are pH-sensitive due to the presence of a Schiff base. In the acidic tumor microenvironment, smaller NPs are released due to the cleavage of the cross-links, thereby limiting drug delivery to the eye. In the second approach, we will synthesize the CB-based VCP degraders using drug design called proteolysis-targeting chimera (PROTAC). These PROTAC molecules can be designed such that it will specifically target VCP but not PDE6 for degradation. These two approaches will overcome current clinical limitations of CB.

Wnt-regulated Macropinocytosis in an Axin Mutation Model of Hepatocellular Carcinoma

Host Campus: Los Angeles

Lead Investigator: Edward De Robertis

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

It is currently emerging that the Wnt pathway, in addition to nuclear beta-catenin stabilization, is a major regulator of endocytosis. We discovered that the Wnt growth factor causes massive uptake and digestion of extracellular proteins. Recent work has shown that this is due to an increase in macropinocytosis, the actin-regulated cell drinking process that engulfs large volumes of liquid into the cellular fluid space.

The present proposal focuses on the function of Axin, a major tumor suppressor that when mutated activates the Wnt pathway in a hepatocellular carcinoma (HCC) model cell line. We found that Alexander HCC cells (ATCC CRL-8024) have robust macropinocytosis that fuels the growth of these avid cells. We established a cell system in which a stable Alexander HCC cell line reconstituted with Axin1-Flag loses Wnt signaling and macropinocytosis, even after 40 years in culture. Wnt signaling is a driver of liver cancer, with beta-catenin stabilizing mutations present in 19% and Axin1 inactivating mutations in 9.6% of HCCs.

In Aim 1, we will characterize this HCC±Axin1 model system with respect to macropinocytosis of nutrients caused by the presence or absence of a single tumor suppressor gene, and study the changes in lysosomal activity resulting from increased macropinocytosis.

In Aim 2, we will study the effect on the metabolome and proteome of the presence or absence of Axin1, in collaboration with Heather Christofk and James Wohlschlaeger. We will also explore in HCC±Axin1 cells the effects of the GSK3 inhibitors Lithium chloride (LiCl) and CHIR99021, which we recently found are sufficient to trigger macropinocytosis and lysosomal activation in other cell lines.

In Aim 3, HCC±Axin1 subcutaneous xenografts will provide a setting for pilot testing of macropinocytosis inhibitors on Wnt-driven tumor growth in immune-deficient NSG mice, in collaboration with the Christofk lab.

The proposed work is relevant to cancer, not only of the liver, but also for many other cancers resulting from Wnt signaling activation. Our cell biological approach focusing on endocytosis could reveal new therapeutic avenues. Funding from CRCC would greatly help cover a gap in funding due to the loss of our generous 25-year support by the HHMI in November 2019.

Control of the Hedgehog pathway by cilium proteins

Host Campus: Merced

Lead Investigator: Xuecai Ge

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

Mutations in the Hedgehog (Hh) signaling pathway leads to various birth defects and tumor formation, including medulloblastoma, a malignant pediatric brain tumor. The transduction of the Hh signaling relies on the primary cilium, a tiny cell surface organelle that functions as the hub of cell signaling. To reveal new cilium proteins related to the Hh pathway, we have conducted a proximity-based proteomic studies, and uncovered novel cilium proteins. In this proposal, we will focus on one of the novel cilium proteins, Numb, to reveal its regulatory mechanism in the Hh pathway and the Hh-related tumor growth.

Previous studies on Numb implicate this protein in asymmetric cell division and regulation of multiple signaling pathways. However, the molecular mechanism of how Numb participates in these cellular processes remains controversial. Our discovery of Numb's localization in the primary cilium may help elucidate the unknown mechanisms. We found that Numb loss of function leads to accumulation of a Hh transcription regulator, Gli3 repressor (Gli3R). For Hh pathway to be turned on, the Gli3R has to be degraded. Numb has been described as an adaptor protein that links target proteins to the E3 ligase, Itch. Therefore, we hypothesize that Numb is required to switch on the Hh signaling by degrading Gli3R. We will test this hypothesis via the following Aims: 1) Determine roles of Numb as an adaptor of ubiquitin ligase for Gli3R; 2) Identify roles of Numb in the proliferation and differentiation of granule neuron precursors in the developing cerebellum; 3) Determine roles of Numb in the formation of medulloblastoma in mouse models. Our results will elucidate a new regulatory mechanism of the Hh pathway and Hh-related tumor formation.

Precision medicine: modeling liver metastases using patient-derived tumors on a microfluid device

Host Campus: Davis

Lead Investigator: Sepideh Gholami

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

For patients with colorectal liver metastases (CRLM), resection of hepatic tumors remains the only option for a potential cure despite high recurrence rates (up to 75%). Cytotoxic chemotherapy alone has shown limited improvement in survival and immunotherapeutics have not shown great success in microsatellite stable (MSS) CRLM. While progress has been made in relevant research models, there is an unmet need to incorporate immune cell interactions into these models. Current ex-vivo oncologic models lack the milieu of non-cancerous components that influence a tumor including: 1) Primary colorectal cells do not survive in 2D cultures, 2) simple organoids do not adequately mimic essential features of the tumor microenvironment (TME) 3) murine models and patient-derived xenografts are costly and lack an intact immune response, and 4) syngeneic mouse models lack a human target and do not facilitate the development of the chronic inflammatory environment characteristic of human tumors, which can stimulate immunologic inhibitory pathways.

The George laboratory has developed a novel autologous ex-vivo “tumor-on-a-chip” model, a microfluidic system of 3D human microtissues, in this case, fresh surgical specimens, perfused with a vascular network that recapitulate salient features of the cancer microenvironment including the relevant immune components. We have further refined and developed this model for patient-derived CRLM. We aim to demonstrate that our ex-vivo 3D chip mimics the immune and TME of CRLM patient avatars and will serve as a platform to study host immune cell-tumor interactions. We will test our hypothesis that MSS, RAS-wildtype (WT) CRLM respond to EGFR inhibitors and demonstrate an immune-rich TME compared to RAS mutant (MUT) tumors on the chip as seen in patients. In Aim 1 we will determine if molecular and immune phenotypes of RAS-WT and RAS-MUT CRLM are preserved on the 3D chip and comparable to surgical specimens. In Aim 2 we will test if ex-vivo responses to targeted therapy of RAS-WT and RAS-MUT CLRM on the 3D chip correlate with the clinical responses noted in individual patients’ tumors.

Leveraging such a system would allow for manipulation of the immune component, a critical step for developing more successful targeted and immunotherapeutic strategies for CRLM in a clinically relevant model.

Synthetic Studies on a FoxO3a Transcription Factor Inhibitor

Host Campus: Los Angeles

Lead Investigator: Patrick Harran

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Chronic myeloid leukemia (CML) is treated effectively with tyrosine kinase inhibitors (TKI). Post therapy, CML recurrence often derives from dormant, drug resistant CML stem cells. Growth of these cells is thought to be driven by the accumulation of nuclear transcription factor FoxO3a. FoxO3a is also critical for the self-renewal of hematopoietic stem cells (HSCs) and leukemia initiating cells (LICs). The FoxO (forkhead box subclass O) family of proteins normally function as tumor suppressors by inducing cell cycle arrest and apoptosis. The FoxO3a transcription factor is a disordered protein that behaves differently. It has been shown to induce cancer cell invasion and metastases as well as drug resistance. Therapies able to eliminate CML stem cells not targeted by TKIs would be highly valuable. The peptidomimetic natural product JBIR-141 was recently discovered as an inhibitor of FoxO3a induced transcriptional activity. It is potently cytotoxic towards SKOV-3, MESO-1 and Jurkat cells in vitro (IC50 = 12, 90 and 4 nM, respectively). The natural abundance of the molecule is low, it has not been synthesized and its molecular mode of action is unknown. The goal of this project is to develop a short, modular synthesis JBIR-141 and thoroughly probe its structure activity relationships. The reactivity and stability of its oxazoline, nitrosamine and acyl tetramate motifs will be explored. We speculate the natural product is a membrane permeant pro-form of an active substance generated intracellularly. Our experiments will address that possibility and set the stage for key collaborative studies of its molecular mode of action and its potential as a new, targeted therapeutic.

Biobehavioral Intervention to Reduce Adverse Outcomes in Young Adult Latinos with Testicular Cancer

Host Campus: Irvine

Lead Investigator: Michael Hoyt

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

Testicular cancer (TC) diagnosis and treatment, especially given its threat to sexuality and reproductive health, can be distressing in young adulthood. In fact, the prevalence of depressive symptoms in TC exceeds the general population, and young Latino men are at high risk for adverse outcomes after treatment. In fact, the majority of young adult cancer survivors will experience impairing, distressing, and modifiable physical, behavioral, and psychosocial adverse outcomes that persist long after the completion of medical treatment. Yet, few targeted, tailored, culturally-relevant interventions exist to assist young Latino survivors in re-negotiating life goals and regulating cancer-related emotions and none focus on reducing cancer burden via biobehavioral mechanisms. Young or “emerging” adulthood is marked by goal attainment. Chronic illness experienced as “off time” in the lifespan interrupts goal pursuits and threatens valued life directions. As young adults return to goal pursuits, re-entry to post-cancer life can be a critical point in the survivorship trajectory. Behavioral intervention at this time is well positioned to confer longer-term impact. Emergent from our group’s preliminary research, we developed and pilot-tested Goal-focused Emotion-Regulation Therapy (GET) as a behavioral intervention to enhance self-regulation through improved goal navigation skills, sense of purpose, and ability to regulate emotional responses in young adults with TC. Responsive to the need for feasible, effective, and scalable interventions that meet the need of ethnic minority men, 25 Latino young adults (ages 18-39) with TC will receive 6 sessions of GET. This pilot study aims to establish feasibility, clinically-meaningful (not statistically significant) change, and guidance for cultural adaptation. We predict that GET, and our ability to detect meaningful change, will be feasible in Latino young adult TC survivors. We expect GET to be associated with reduced distress and reductions in adverse biobehavioral indicators (dysregulated stress hormones, elevated inflammation). We also expect that greater endorsement of culturally-relevant factors (i.e., familism, simpatia, acculturation/acculturative stress, machismo/caballerismo) will condition the impact of GET on primary and secondary outcomes and that qualitative data will identify culturally-relevant adaptation.

Epigenetic landscape of DNA methylation in pancreatic cancer progression

Host Campus: San Diego

Lead Investigator: Chang-il Hwang

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

Pancreatic cancer is one of the deadly human malignancies, owing in part due its early onset of metastasis. Key driver mutations for pancreatic cancer metastasis have not been identified, and alteration of epigenetic pathways has been suggested as a potential mechanism. We have established the innovative pancreatic cancer organoid cultures derived from both patients and genetically engineered mouse models. Pancreatic organoid cultures are amenable for genetic manipulations and suitable for high throughput 'omics' approaches, allowing us to dissect the underlying molecular mechanisms. Using the pancreatic organoid models, we have reported that epigenetic reprogramming as a potential factor in pancreatic cancer metastasis. Two major epigenetic changes are histone modifications and DNA methylation. Previously, we have extensively profiled the key histone modifications in pancreatic cancer organoids. However, another key epigenetic regulation, alterations of DNA methylation landscape remains to be determined in pancreatic cancer progression. Here, we propose to profile genome-wide DNA methylation in both human and mouse pancreatic organoid models and identify functionally important epigenetic changes of DNA methylation in metastasis. We have performed the Reduced Representation Bisulfite Sequencing (RRBS) in the murine organoid models for pancreatic cancer. We will investigate how DNA methylation pattern changes in pancreatic cancer progression (normal, PanIN, tumor and metastasis). In addition, we will intersect DNA methylation data with transcriptional profiles and key histone modifications in the promoter and enhancer regions. This will provide mechanistic insights in how differential DNA methylation contributes to gene expression and disease progression. To determine the effect of the differential DNA methylation in individual genes, we will employ CRISPR-dCas-DNMT or -TET system to methylate or demethylate specific regions of interest, respectively. This will enable us to identify functionally important DNA methylation for pancreatic cancer metastasis. In sum, the proposed study will elucidate the epigenetic landscape of DNA methylation in pancreatic cancer progression using pancreatic organoid models. Furthermore, genes associated with differentially methylated regions can be exploited for the development of novel diagnostic and therapeutic strategies.

Histamine H1 (HRH1): A novel therapeutic target for pancreatic ductal adenocarcinoma?

Host Campus: San Diego

Lead Investigator: Paul Insel

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$60,953

Abstract:

In 2020, it is estimated that ~57,000 people will be diagnosed (and ~47,000 will die) with pancreatic ductal adenocarcinoma (PDAC), the most common type of pancreatic cancer. PDAC has a 5-year survival of ~9%. Within the next few years PDAC is predicted to be the second leading cause of cancer death. Progress in the treatment of PDAC has thus lagged behind that of many other cancers. My laboratory has an unconventional idea to address this important unmet, medical need. We have asked: Might it be possible to target certain cell surface binding sites, termed receptors, in particular G-protein-coupled receptors (GPCRs, the largest class of targets for approved drugs) by re-purposing FDA-approved GPCR-targeted drugs to aid in the treatment of PDAC? This project will test whether a particular GPCR, one that binds histamine (known as HRH1), which we have discovered is highly expressed in PDAC, influences PDAC cell function and if its actions can be blocked by FDA-approved HRH1 inhibitors (antihistamines). If so, such antihistamines could potentially be re-purposed as an effective (and likely safe) way to treat PDAC patients.

Our preliminary studies, using 1) analyses of data for PDAC tumors in The Cancer Genome Atlas (TCGA) and for normal human pancreas in another database and 2) studies with human PDAC cell lines and PDAC cells have identified GPCRs with much higher expression in PDAC. HRH1, among the highest such GPCRs, has many FDA-approved inhibitors (antihistamines, typically used to treat allergic conditions). We hypothesize that HRH1 antihistamines may be candidates for drug re-purposing to treat PDAC. Our Specific Aims will ask: Aim 1) Does histamine, which activates HRH1, alter the function of human and mouse PDAC cells and if so, do FDA-approved antihistamines block such effects? Aim 2) Do FDA-approved HRH1 antihistamines improve the action of agents approved for the treatment of PDAC. These latter studies will test whether FDA-approved antihistamines will enhance the killing of PDAC cells by drugs currently approved and used to treat patients with PDAC. Success in the proposed studies could help set the stage for the rapid initiation of clinical trials with one or more antihistamines in combination therapy as a new approach for the treatment of PDAC and that might improve its currently dismal outcome for PDAC patients.

Intron Retention in Nutrient Response and Cellular Quiescence

Host Campus: Los Angeles

Lead Investigator: Tracy Johnson

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

There are a number of well-characterized changes in cell behavior that are hallmarks of cancer. These include changes in cellular metabolism and bypass of signals that usually suppress cell growth. An important step in understanding cancer is understanding the molecular basis of these events.

In order to address this challenge, we are leveraging the model eukaryote *Saccharomyces cerevisiae* as an experimental system. With its streamlined genome and powerful molecular genetic tools, yeast cells lend themselves well to detailed molecular analyses. Not surprisingly, there is a long history of cancer-relevant molecular mechanisms that have been elucidated from yeast studies.

In yeast, as with other eukaryotic organisms, more core crucial processes are regulated by messenger RNA splicing, the removal of non-coding sequences, introns, from protein coding genes. Intron removal is a critical step in producing an RNA that is a viable substrate for protein synthesis. Mis-regulation of splicing is a significant contributor to tumorigenesis. However, under a variety of conditions—both in normally functioning cells and in disease states, introns are “retained.” The fate and function of these retained introns are poorly understood.

We have recently found that the ability of yeast cells to adjust their metabolism in response to glucose availability is dependent upon regulation of intron retention. In fact, both glucose metabolism and cellular quiescence are regulated by intron retention. Surprisingly, some intron-retained transcripts contain signals within the intron that allow the production of a functional protein product. The following aims explore the nature of these signals and their effect on cell growth and glucose metabolism.

Aim 1: Determine what pre-mRNA features affect intron retention in response to glucose availability.

Aim 2: Determine what intronic features contribute to recognition of intronic translation signals.

Aim 3: Characterize proteins that facilitate recognition of intronic translation signals.

In light of the abundance of introns, the prevalence of their retention in mammalian cells, and the conservation of proteins involved in both splicing and translation, these studies will address fundamental mechanisms of gene expression that affect cellular function and dysfunction, including important hallmarks of cancer.

Antileukemic organoarsenicals as safe and effective alternatives to arsenic trioxide

Host Campus: Santa Cruz

Lead Investigator: Timothy Johnstone

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

Arsenic trioxide (ATO) is an FDA-approved frontline therapy for acute promyelocytic leukemia (APL). Past mechanistic studies have shown that ATO functions by crosslinking aberrant PML-RAR α fusion proteins that are characteristic of APL. The activity of these fusion proteins prevents the differentiation of the mutant promyelocytes, which ultimately leads to the disease phenotype. As(III) centers derived from ATO crosslink the proteins through cysteine thiolates, leading to protein aggregation and promyelocyte differentiation. Unfortunately, however, the As(III) released by ATO indiscriminately binds to cysteine thiolates throughout the cell and the organism at large, which contributes to significant off-target toxicity. The proposed research project involves the design, synthesis, and testing of arylarsonic acids, which have the general formula RAsO(OH)₂. Organoarsenic species are less toxic than inorganic arsenic compounds, like ATO, and the structure of the R group can be systematically tuned to increase cell uptake and specificity for binding to PML-RAR α . Moreover, arsonic acids feature arsenic in the less reactive and less toxic 5+ oxidation state, as opposed to the 3+ state found in ATO. These prodrugs can then be reduced in the intracellular environment to achieve the reactive 3+ oxidation state. Our laboratory has experience in the synthesis, characterization, and testing of medicinal inorganic compounds, but the proposed project is at the fundamental conception stage and funds are requested to cover the costs of chemicals, cell culture supplies, instrument recharge, and trainees. This funding would allow us to generate the preliminary results needed prepare a competitive application for a major federal operating grant.

Role of a novel RNA endonuclease EndoU in squamous cell carcinomas

Host Campus: Riverside

Lead Investigator: Fedor Karginov

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Squamous cell carcinomas (SCCs) are the most common solid tumors, imposing an enormous mortality burden worldwide. This group includes lung, esophagus, oral cavity, skin, cervical and vaginal tumor types. All such carcinomas originate from the unchecked proliferation of stratified epithelial cells, and share common mechanisms of development and progression. We have identified the EndoU gene to be implicated in SCC as a novel potential tumor suppressor. EndoU is strongly downregulated in cervical, esophageal and oral squamous carcinomas, and its expression correlates with favorable prognosis in the squamous-epithelial head and neck cancers, as well as colorectal tumors, underscoring the significance of this factor in important developmental and disease processes. These observations are consistent with a known role for EndoU in promoting cell death during B cell development; however, its function in SCCs is entirely unknown. The EndoU protein is an RNA endonuclease that may post-transcriptionally regulate gene expression programs relevant to proliferation and cell death. Cervical cancer provides an attractive system to investigate the role of EndoU because of its high expression in the cervix lining, strong negative association with tumor development, and the availability of new transgenic mouse models. In this proposal, we aim to determine the tumor-suppressive role of EndoU in cervical cancer using existing mouse models for EndoU KO and cervical cancer establishment, as well as cell line models to investigate the expression and contribution of EndoU to cell proliferation and death. Successful completion of this proposal is very likely to shed light on the broader role of EndoU in other squamous cell carcinomas. Specifically, the results would support the investigation of EndoU in esophageal cancer (where robust transgenic mouse models are not available), as well as in oral cavity, lung, and vaginal squamous cell carcinomas. In addition, despite the absence of stratified epithelia in the colon, the strong prognostic power of EndoU in colorectal cancers may prompt its investigation in this prevalent cancer type.

Germline DNA-based Biomarkers of Toxicity Following Prostate Radiotherapy

Host Campus: Los Angeles

Lead Investigator: Amar Kishan

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Prostate cancer (PCa) is the most common solid malignancy among men in the United States and a major cause of morbidity and mortality worldwide. Curative intent external beam radiation therapy (RT) for PCa has been historically delivered in the form of low daily doses, or fractions, of RT over a total of 39 to 45 fractions. Because PCa cells appear to be preferentially killed by higher doses of RT per fraction (even if to a lower total overall dose), the modern technique of stereotactic body radiotherapy (SBRT) allows the treatment course to be condensed to as short as five fractions. However, while the rates of significant genitourinary and gastrointestinal toxicity are similar between SBRT and longer RT courses, it is unknown which patients are predisposed towards developing significant toxicity following either treatment. Given the higher doses per day delivered with SBRT, trepidation towards its use persists.

Variants in germline DNA (i.e., DNA that is inherited and intrinsic to any given person) are thought to strongly influence a given patient's response to RT. We have significant data from a translational study of over 200 men that suggest that biomarker panels defined by single nucleotide polymorphisms in germline DNA predict significant genitourinary toxicity after longer-course RT or SBRT with a high level of accuracy. A practically relevant aspect of examining germline DNA is that any type of biological sample taken from a patient, such as an easily obtained cheek swab, would contain enough material for analysis.

The project being proposed for a New Assistant Professor Faculty Seed Grant from the University of California Cancer Research Coordinating Committee is an innovative prospective clinical trial termed the "Germline DNA-Based Radiosensitivity Biomarker Influence on Decisions About and Toxicity Following Prostate Radiotherapy" (GARUDA) trial. This trial is designed to validate the germline predictors of toxicity we have identified, thereby establishing the ability to identify predictors of radiotherapy before RT. If validated in this prospective study, these germline predictors would have significant implications for the large number of patients who are pursuing RT for their PCa. Specifically, it would increase the confidence of both the patient and the physician that the expected rate of significant toxicity would be low.

Synthesis of Benzylisoquinoline Alkaloids as Antimitotic Agents for Cancer Therapy

Host Campus: Riverside

Lead Investigator: Kevin Kou

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

The isoquinoline alkaloids are naturally-occurring small molecules that suppress the proliferation of lung, breast, and leukemia cancer cell lines, including those that express mutant-p53. Their antiproliferative and proapoptosis functions are believed to arise from mechanisms of action that interfere with microtubule networks and p53 proteins. Thus, this proposal seeks to develop novel therapeutic leads to combat cancer. Inspired by the antimitotic function of scoulerine and berberine, we will synthesize structurally-related natural products within this family that have never been subjected to bioevaluation so that a thorough understanding of their antiproliferative effects can be advanced. Computational modeling will be employed to gain insight on substrate-protein interactions. It is our intention to use this knowledge to design and synthesize a series of non-natural analogs with improved potency and investigate structure-activity relationships through cytotoxicity screening and proliferation/viability assays. The goal of these studies is to develop novel lead candidates for cancer therapy.

Exploring the functions of conserved long non-coding RNAs in cancer cell growth

Host Campus: San Diego

Lead Investigator: Colleen McHugh

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$84,750

Abstract:

While each human cell contains all the DNA required to create an entire organism, only a subset of these genes are expressed in each individual cell. Aberrant transcriptional expression and epigenetic changes are common in cancer cells compared to normal cells. Long non-coding RNAs (lncRNAs) have recently captured the interest of many researchers due to their ability to act as regulators and organizers of gene expression in the cell. We have identified a set of five conserved long non-coding RNAs required for cancer cell survival, based on data mining of screens for cancer associated long non-coding RNAs, and several published CRISPR-Cas9 screens of thousands of non-coding RNAs. Some of these lncRNAs have been studied previously in the context of cancer metastasis and progression, but the pathways in which they function are still unknown.

We have developed a powerful mass spectrometry screen for specific lncRNA interacting factors that enables the identification of direct, zero-distance interactors with an RNA of interest. This method has already been successfully used to characterize the interacting factors of Xist lncRNA in embryonic stem cells, but no cancer related experiments have so far been performed. The experiments in this proposal will extend the method to cancer cell related lncRNAs, with the potential to discover a new set of regulatory roles for non-coding RNAs.

Using this seed funding, we will explore the molecular basis for the observed cell growth phenotypes by establishing a knockdown and overexpression system for each of the five conserved lncRNAs. We will characterize the protein interactome and transcriptional changes induced by overexpression or deletion of each of these five conserved lncRNAs. Since many lncRNAs act as epigenetic regulators, it is anticipated that new signaling pathways for cancer cell growth may be revealed in this study. We plan to apply for external funding to continue to support this project, using the preliminary data gathered during the one-year period of the Cancer Research Coordinating Committee award.

"Lymphomizing" Treatment of Head and Neck Cancer using Involved Field RT with Chemo-Immunotherapy

Host Campus: San Diego

Lead Investigator: Loren Mell

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$82,500

Abstract:

Current standard treatment for most advanced head and neck cancers involves combined chemotherapy and radiation therapy. Newer treatment approaches are incorporating immunotherapy, specifically PD1:PD-L1 checkpoint inhibitors, into the standard treatment paradigm. Standard radiation fields cover both the primary tumor plus "elective" radiation to areas of potential regional spread, especially to lymph nodes, even though many patients do not actually have disease present in the nodes. Large radiation fields result in significant acute and long-term morbidity, particularly difficulty swallowing, which can lead to feeding tube dependence and risk of aspiration. In the setting of concurrent immunotherapy, it is not clear that such large fields are required. For example, in the treatment of lymphoma, successive advances in the quality of systemic therapy have permitted both lower radiation doses and, importantly, smaller field sizes (i.e., Involved Field Radiation Therapy, IFRT), drastically decreasing the morbidity of radiation therapy. Moreover, treatment of healthy nodes with radiation could actually be harmful by interfering with the effectiveness of immunotherapy, which is trying to stimulate the anti-tumor immune response and depends on functional lymph nodes. However, prospective trials are needed to confirm that it is safe to treat head/neck cancer patients with IFRT and that this does not lead to excessively high rates of recurrence, which to our knowledge has not been tested. Therefore we propose to treat a pilot cohort of 12 patients with IFRT (radiation directed only at visible disease on positron emission tomography) plus immunotherapy with standard chemotherapy (tri-weekly cisplatin), and prospectively assess for rates of disease recurrence. Secondly, we will collect novel information on swallowing function using quantitative imaging techniques in collaboration with speech and language pathology, and will collect serial blood specimens for comparison with data from previous trials using standard radiation.

Investigating metabolic mechanisms promoting growth of primary and metastatic disease

Host Campus: San Francisco

Lead Investigator: Jean Nakamura

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Cancers commonly dysregulate metabolic processes to support growth. A tumor cell's ability to modulate its metabolites influences multiple key aspects of a tumor's behavior, such as cellular signaling, differentiation and metastatic potential. Although the concept of targeting metabolic vulnerabilities has appeal from a therapeutic standpoint, delineation of critical vulnerabilities defined by both gene and specific metabolic context remains a barrier. Using a novel ATP-sensor that enables FACS-mediated isolation of cells on the basis of intracellular ATP levels, we performed CRISPRi screens to identify ATP-modifying genes operating under specific metabolic contexts in human cancer cells (manuscript under revision for Nature Communications). These studies revealed candidate molecules that function as critical participants in tumor metabolic reprogramming. We then expressed our CRISPRi library in multiple human lung cancer cell lines and performed an in vivo growth screen, which identified a class of genes as being critical to growth in multiple in vivo tumor models. Notably, these genes are not well-characterized functionally or linked to cancer biology. However our data suggest important new roles for this class of genes, and this proposal, which represents a new area of study for our laboratory, we will test top candidate genes in pre-clinical studies using a novel in vivo tumor paradigm.

New mouse model of immunosuppressive TGF-beta function

Host Campus: San Francisco

Lead Investigator: Stephen Nishimura

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Non-small cell lung cancer (NSCLC) kills more people in the US than any other cancer. Immune checkpoint inhibitors alone or in combination with chemotherapy in patients with advanced disease has improved survival in numerous studies. However, the majority of patients progress within one year and only a small minority of patients with metastatic disease have long term survival. Thus, there remains an urgent unmet need for novel therapeutic approaches to improve outcomes for patients who have cancer refractory to FDA approved immune checkpoint therapies.

The TGF- β signaling pathway has broad effects on the tumor immune environment (TME) that could contribute to resistance to immunotherapy. Extensive preclinical studies indicate that TGF- β establishes an immunosuppressive TME. Recent preclinical studies have demonstrated that inhibiting TGF- β signaling restored tumor response to checkpoint inhibition in mice. However, the ultimate utility of targeting TGF- β is limited by toxicities, which have been documented in rodents, primates and humans using global TGF- β inhibitors.

It would be a highly significant advance to establish the mechanistic basis of TGF- β mediated immunosuppression to understand how to most efficaciously target TGF- β function in NSCLC and other cancers. Such mechanistic understanding could lead to both more selective inhibition of TGF- β function, and limit risk of toxicity. We have found that increased selectivity can be achieved by focusing on TGF- β activation, since TGF- β is expressed in a latent form (L-TGF- β) complex that must be activated in order to function. Using a structural biologic approach, we have proposed a new model of TGF- β activation where TGF- β signaling occurs within the L-TGF- β complex without release and diffusion of TGF- β . This model predicts that within the tumor microenvironment that tumor cells can activate TGF- β signaling in contacting immune cells. This mechanism provides a precise mechanism of TGF- β activation to target for immunotherapeutic benefit. Here we will establish a genetic mouse model where TGF- β can only be activated and signal by this highly specific mechanism. This model will be used to establish the relevance of this TGF- β activation mechanism to lung cancer immune evasion and to establish methodologies to more precisely and efficaciously target it.

Supramolecular Cyclodextrin Frameworks for immunoprotein immobilization

Host Campus: Irvine

Lead Investigator: Joe Patterson

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Over the past century, tumor treatment has predominantly focused on radio and chemotherapy. Since the beginning of the new millennium, new treatments have been developed using immunotherapy, which enhances the immune response of the body against cancer. Many types of proteins, such as antigens, antibodies and immunomodulators, can be used to create an immune response and have been gaining interest in the field of research, diagnosis and treatment of cancer. Despite the remarkable results in the lab, the application of immunoproteins has been limited by their low structural stability, propensity to aggregate, and degradation by enzymes. Significant efforts have been made to eliminate these effects through immobilization of the immunoproteins onto a matrix, however, no matrix currently exists which tackles all of these limitations. In this project, we will address all of the limitations using a combined matrix approach, that's uses cyclodextrin (CDs) and metal-organic frameworks (MOFs). Cyclodextrins-proteins complexes, have been shown to reduce protein aggregation and provide higher structural stability to physical perturbation. MOFs have been shown to provide protection against other enzymes, increase protein activity and provide control of the delivery of their carrying molecules. However, both individual matrices present limitations, the CD approach only provides short term storage and are not protective against enzymes. The MOF approach only provides structural stability for a very limited number of enzymes which are compatible with the MOF pores and is not widely applicable to immunotherapy. Despite the fact that a combined CD-MOF approach to immunoproteins immobilization is a logical way to accelerate their use in cancer treatment, there have been no current efforts to do so. In this project we will develop a simple immobilization strategy for immuno-related proteins using a combination of CDs and MOFs. Specifically, the CD-MOF will be designed so that the CD and MOF will serve as a synergistic structural shield against any type of physical, chemical and biological perturbation.

A polygenic score for prediction of aggressive and fatal prostate cancer in multi-ethnic populations

Host Campus: San Diego

Lead Investigator: Tyler Seibert

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

When detected in its early stages, prostate cancer is curable, but it still causes over 350,000 deaths per year. Screening with a blood test to measure prostate-specific antigen (PSA) leads to earlier detection and has been shown to reduce cancer deaths. However, PSA testing of the entire male population leads to many false positives and many diagnoses of slow-growing cancers that are unlikely to cause significant problems. A test is needed to help physicians decide whether a given patient would benefit from screening—and what age to start that screening. Such personalized decisions might be made by measuring each man’s genetic risk for aggressive forms of prostate cancer. A genetic score (called a polygenic hazard score, or PHS) has been developed and tested in a dataset of thousands of men. The PHS was strongly associated with aggressive prostate cancer. While promising, the PHS was developed and validated with only data from men of European ancestry, reflecting data availability at the time.

We have obtained access to a large, multi-ethnic dataset, through collaboration with the international PRACTICAL consortium. We propose here to test the original PHS to see whether it performs well in a new dataset and whether performance is affected by race/ethnicity. Rather than self-reported race/ethnicity, we will use genetic ancestry (European, African, or Asian) determined by the individual’s own DNA. We will evaluate whether PHS is associated with age at diagnosis of aggressive prostate cancer and with lifetime risk of death from prostate cancer. PHS will be compared to family history and other risk factors for prostate cancer. Our preliminary results suggest that the original PHS works in men of multiple racial/ethnic backgrounds, but performance is best in genetic Europeans.

After formally testing the original PHS in each genetic subgroup, we will use the multi-ethnic data to optimize the score for each genetic ancestry. We will do this by searching for genetic markers within each genetic ancestry that are associated with age of onset of prostate cancer and incorporating them into an enhanced PHS.

Ultimately, PHS could guide personalized prostate cancer screening decisions for men of all races/ethnicities, thus saving lives through early detection.

The genomic landscape of cutaneous squamous cell carcinoma

Host Campus: San Francisco

Lead Investigator: Alan Shain

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Cutaneous squamous cell carcinoma (cSCC) is a form of skin cancer, originating from keratinocytes, that kills an estimated 8000 people per year in the United States. Compared to other cancers with similar death tolls, our understanding of the somatic mutations driving cSCC is limited. Comprehensive genomic studies of other tumor subtypes have markedly accelerated drug development, opened new avenues of treatment, and revealed fundamental insights into their basic biology – similar caliber studies are warranted for cutaneous squamous cell carcinoma. The overarching goal of this grant is to define the driver genes in cutaneous squamous cell carcinoma. cSCCs were not included in large-scale sequencing initiatives, such as The Cancer Genome Atlas (TCGA) program, but there are 9 small-scale studies, cumulatively encompassing 162 tumors, with exome or genome sequencing data. For this grant, we will perform a meta-analysis, aggregating and reanalyzing the data from these smaller studies. We will also supplement this data by additionally sequencing the exomes of 27 tumors from our own institution. In aggregate, our analysis will be nearly five times the size of the largest individual study to date. Aside from the small size of most studies, another obstacle to cancer gene discovery is that cutaneous squamous cell carcinomas have high mutation burdens, primarily from UV radiation, making it difficult to distinguish driver mutations from the overwhelming number of passenger mutations in these tumors. Not long ago, the melanoma community was afflicted with similar challenges, and I was at the forefront of efforts to overcome this challenge. Here, we will invoke similar strategies by applying state-of-the-art cancer gene discovery tools to identify the driver genes in cutaneous squamous cell carcinoma. The research proposed here is well-matched for this award mechanism because it will produce a rich resource of hypothesis-generating data to support future grants. Moreover, this grant is feasible for an award of this size in large part because it leverages a resource of pre-existing data. In closing, genomic studies have proven fundamental in advancing our understanding of other cancers – here, we will address this gap in knowledge for cSCC.

Label free, high throughout detection and separation of individual breast cancer stem cells

Host Campus: Irvine

Lead Investigator: Zuzanna Siwy

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Cancer stem cells (CSCs) are a rare cellular subset within a tumor (1-5% but in some tumors only up to ~0.01% of the total tissue) that are believed to be responsible for the metastatic progression of cancer, as well as resistance to chemotherapy and radiation therapies, and disease relapse. As such, the successful isolation of viable CSCs with minimal perturbation and manipulation, would enable further understanding of CSC biology, which is necessary for the development of novel therapeutics directly targeting this rare subset of aggressive cells. This is especially relevant to breast cancer where 100% of mortality is due to metastasis. The goal of the proposal is to design a microfluidic platform capable of label-free isolation of viable CSCs breast cancer cells from blood and tissue.

CSCs will be separated based on their unique mechanical properties. Identifying the cells using physical and mechanical properties rather than chemical markers makes the platform independent of an a priori knowledge of cells' surface characteristics, which is often unknown. The microfluidic channel we designed contains a cavity flanked by two narrower regions; at all positions along the channel, the channel width is larger than or comparable to the cells' size. The inhomogeneous pressure gradients and shear stress in such a channel cause multiple deformations of the cell in both directions. The new platform will provide characterization of individual cells and allow finding one-to-one correspondence between a given cell's mechanical properties and the same cell's biological function. Hundreds of cells will be analyzed per second, and 5 mL of cells suspension will be analyzed within 15 minutes.

In Aim 1, the cells (MCF-7 and MDA-MB-231) will be characterized by combined optical and electrical signals analyzed by advanced machine learning approaches. Machine learning algorithms will be applied to find direct correspondence between the optical and electric signals, enabling one to characterize cells' deformation based on electrical recordings only.

In Aim 2 we will develop a platform to isolate individual CSCs that can be subjected to further biochemical analysis.

This work will lay the foundation toward the understanding of CSCs, the cancer stem cell model, and the development of potential CSC-specific drug therapies for the treatment of metastatic cancer.

Investigation of microenvironmental changes to the bone marrow after cytotoxic conditioning

Host Campus: Merced

Lead Investigator: Joel Spencer

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

The bone marrow microenvironment, or niche, houses cells, extracellular matrix, and a milieu of factors that help maintain and regulate hematopoietic stem and progenitor cell (HSPC) function in order to produce sufficient hematopoietic cells over the lifetime of an individual. One of the molecules thought to play an important role in HSPC regulation is bimolecular oxygen (O₂). In many pathological conditions such as cancers and inflammation, and in therapeutic regimens such as radiotherapy or chemotherapy, bone marrow oxygenation is disrupted. For example, aberrations in bone marrow hypoxia (low oxygen levels) is a known characteristic of acute leukemias including in patients and evidence suggests that hypoxia or signaling through hypoxia inducible factor (Hif-1), a master regulator of the hypoxic response in cells, may induce spontaneous metastasis to the bone marrow and promote tumor colonization in other cancers (e.g., breast cancer). Furthermore, tumor hypoxia remains a significant barrier to radiotherapy, chemotherapy, and immunotherapy in cancer treatment. We previously determined that elevated levels of oxygen (hyperoxia) are found in the bone marrow 3 days after lethal irradiation (9.5 Gy), sublethal irradiation (4.5 Gy), and chemotherapy (busulfan) due to increased blood flow and decrease oxygen consumption. By day 5 after transplantation of whole bone marrow cells, however, the oxygen landscape begins to change with specific locations experiencing extreme hypoxia (<1% oxygen) while other areas still experiencing hyperoxia. How pathologic disruption of bone marrow oxygenation occurs and the role that hypoxia plays in therapeutic efficacy of cancer is not fully understood. In this proposal, we aim to investigate the microenvironmental changes to the niche (e.g., hemodynamics, vascular remodeling, and oxygenation) over time after clinically relevant cytotoxic conditioning regimes used to treat hematologic malignancies. We will directly visualize these changes over time using intravital two-photon microscopy of the mouse bone marrow in both acute myeloid leukemia models and in cancer free controls with the goal of understanding how and when these therapies disrupt the microenvironment. This proposal will provide crucial preliminary data for a future R01 proposal and may also be sufficient for a small manuscript.

Elucidating Heterogeneity in the Microenvironment of Colorectal Cancer at Single-Nucleus Resolution

Host Campus: San Francisco

Lead Investigator: Robert Warren

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

Metastatic colorectal cancer (mCRC) is common, yet therapies are limited, and the 5-year survival rate is dismal. Bulk gene expression profiling has enabled classification of localized CRC into molecular subtypes predictive of clinical behavior. However, the prognostic value of subtyping in metastatic CRC is less clear. Since it is metastases that kill most patients, the need to characterize mCRC is compelling. Further, many genes associated with poor-prognosis subtypes are expressed by stromal rather than neoplastic elements such as cancer-associated fibroblasts (CAFs). Since cellular diversity within a tumor is poorly captured by bulk transcriptomics, we propose that single-nucleus gene expression analyses of mCRC tumors can identify stromal cell types associated with clinical outcome.

My group has generated a unique biorepository of cryopreserved hepatic metastases from >200 patients undergoing resection for mCRC and has established the feasibility of single-nucleus RNA sequencing (snRNA-seq) on snap-frozen liver metastases. Preliminary findings support the hypothesis that stromal cell populations, particularly CAFs, comprise a biologically important component of mCRC tumors. While the high cost of snRNA-seq has limited its applicability to large patient studies, our co-investigator at UCSF, Dr. Zev Gartner (Pharmacological Chemistry) has developed a method for multiplexing samples for massively parallel single-nucleus transcriptional analyses, with a marked cost savings.

We will profile the transcriptional state of tumor and stromal cells in mCRC liver metastases from 50 patients and characterize chemotherapy-induced changes in the stromal and tumor transcriptome by comparing tumors from chemo-naïve and -treated patients. We expect this to reveal insights into the mechanisms of sensitivity or resistance to first line chemotherapy in patients. Since KRAS mutations are strongly associated with poor outcome in mCRC, we will investigate impact of RAS mutations on the potential interplay between cancer cells and stromal cells in mCRC through transcriptomic analysis of patient tumors. Our goal is to elucidate how tumor and stromal cells interact to define the phenotype of mCRC, forming the foundation for an NIH proposal, in which stromal cells will be added to tumor organoids to engineer a more predictive in vitro model of patient-specific drug responses.

Molecular basis of force-sensing by keratin network

Host Campus: Davis

Lead Investigator: Soichiro Yamada

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$85,000

Abstract:

Physical integrity of epithelial tissue is established and maintained by the cytoskeletal network that integrates neighboring cells and extracellular matrix. In particular, keratin intermediate filament proteins expressed in all epithelial tissues are thought to be responsible for structural integrity of epithelial tissues and recently emerged as a driver of collective cell migration. Yet, unlike actin, understanding of keratin mechano-biology is still very limited. In this proposal, we demonstrate that keratin network responds to externally applied physical forces by recruiting cten, a protein known to act as both a tumor suppressor and promotor in a tissue specific manner. We hypothesize that force-dependent interactions surrounding cten are responsible for opposing functions of cten in various cancer cells. Therefore, our goal is to determine the differences in force-dependent protein interactome surrounding cten in various cancer cell lines. To identify force-sensitive protein-protein interactions surrounding cten, the protein interactions are detected by in situ proximal biotin labeling while cells are being physically stimulated. By fusing cten to a promiscuous biotin ligase, any proximal proteins are biotinylated. The biotinylated proteins are identified by proteomic screening using mass spectrometry analysis and the degree of biotinylation in the presence or absence of external force will be used to classify force-sensitive protein interactions. Newly identified force-sensitive protein candidates will be tested to verify their force-dependent co-localization with cten and keratin filaments in various cancer cells. This approach will reveal the comprehensive list of cten associated proteins in the presence or absence of external forces in cancer cell lines and may explain opposing functions of cten in various cancers.

Targeting energy metabolism for cancer control

Host Campus: Irvine

Lead Investigator: Kyoko Yokomori

Start Date: 10/1/2020 *End Date:* 9/30/2021 *Amount:* \$75,000

Abstract:

Cancer metabolism and its therapeutic manipulation are important areas of cancer research. A major characteristic of cancer cells is their increased reliance on glycolysis to maintain cellular homeostasis (Warburg effect). Interestingly, however, cancer stem cells were shown to have a unique metabolic flexibility to switch back to oxidative phosphorylation (oxphos), which is critical for their maintenance. Furthermore, the oxphos pathway is required for migration and metastasis of malignant cancer cells. These results collectively support the notion that despite the well-appreciated Warburg effect, the oxphos pathway remains critical for cancer cell survival, and may be a promising therapeutic target. Mitochondrial oxphos inhibitors were shown to have anti-cancer activity but their toxic side effects have prevented their use in cancer treatment in the past. Our recent study indicates that cancer cells increase their metabolic reliance on oxphos in order to survive in the presence of DNA damage when poly(ADP-ribose)ase 1 (PARP1) is activated, and that the normal cells appear to be relatively resistant to this possibly because of their high basal oxphos level. Thus, we hypothesize that by understanding and manipulating the metabolic response to DNA damage, increasing the cancer cell sensitivity to mitochondria oxphos inhibitors with minimum side effect on non-cancerous cells may be possible. While PARP inhibitors are effective anti-cancer drugs, the emergence of PARPi-resistant cancers is becoming a problem. Our strategy to activate PARP (rather than to inhibit PARP) may provide an effective alternative treatment option in these resistant cancers. Thus, our goal is to further our understanding of dynamics of cancer metabolism, and explore a novel effective cancer treatment method. Specific Aims are (1) to determine how general this sensitivity to perturbation of oxphos in response to DNA damage among different cancer types, and (2) to address the underlying mechanism of sensitivity and resistance using genetic screening. The successful outcome of this project may provide new insight into cancer metabolism and lead to possible discovery of novel therapeutic targets and potentially effective treatment for difficult-to-treat cancers.