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Radiation and cognitive dysfunction: Role of Adenosine

Host Campus: Irvine
Lead Investigator: Munjal Acharya
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Cranial irradiation (IRR) during the clinical radiotherapy of cancer may elicit early onset or more severe cognitive dysfunction. Pediatric cases are of particular concern, as children can lose up to 3 IQ points/year and often live long lives post-cancer. Cognitive domains affected involves a range of neurodegenerative effects including a decline in neurogenesis, oxidative stress, compromised neuronal structure and astrogliosis. However, the molecular and cellular mechanisms underlying IRR-induced cognitive decline have not been resolved. Astrocytic networks in the brain provide metabolic clearance of surplus neurotransmitters and signaling metabolites like adenosine. Extracellular adenosine is tightly regulated by astrocytic adenosine kinase (ADK). ADK overexpression during astrogliosis depletes adenosine leading to enhanced excitation in the brain. Even a subtle change in ADK can rapidly translate into major drop in adenosine that may significantly impact neuronal function, suggesting a neuroprotective role for adenosine. We hypothesize that altered adenosine/ADK dynamics play important roles in the perpetuation of IRR-induced cognitive dysfunction. Our preliminary data indicate that ADK inhibition via systemic (i.p.) 5-iodotubercidin treatment can ameliorate cognitive impairments in rats exposed to cranial IRR (10 Gy) long-term, providing the first evidence that therapeutic manipulation of adenosine homeostasis can effectively ameliorate IRR-induced neuropathology. These results indicate a novel mechanism where adenosine augmentation can exert neuroprotection against radiation-induced CNS damage. Correspondence of elevated ADK expression and astrogliosis post-IRR indicate a critical role of the astrocytic compartment in the regulation of the extracellular adenosine pool. To understand better the mechanistic regulation of astrocyte-mediated adenosine/ADK modulation, we propose to utilize our well established molecular tool – ADK knockdown (Adk-KO) vector – to study the CNS-radiation response and its impact on cognitive function. Availability of AAV8-based Adk-KO vector targeted to astrocytes affords selective knockdown (80%) of ADK and a corresponding increase in adenosine, thereby providing a useful and selective tool to mechanistically examine the importance of adenosine/ADK in the pathology of radiation-induced cognitive dysfunction. This proof-of-principle study will lay the foundation for novel astrocyte-specific therapeutic interventions to curtail cranial radiation-induced cognitive dysfunction.

Integrating immunotherapy with targeted chemo-radiotherapy

Host Campus: San Diego
Lead Investigator: Sunil Advani
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Classically, radiotherapy is utilized as localized cancer therapy and in patients with locally advanced cancers, the most successful therapeutic strategies deliver radiotherapy in combination with chemotherapy. Interestingly, there is accumulating evidence for a systemic role for radiotherapy in the metastatic setting through an abscopal effect, where focal irradiation to one discrete tumor results in tumor responses in distant non-irradiated tumors. The abscopal effects are in part mediated by ionizing radiation (IR) modulating the immune system. This has resulted in clinical evaluation of IR with immunotherapies. However, there is a paucity of studies evaluating immunotherapy in the context of concurrent chemotherapy and IR. A key rationale for combining chemotherapy and radiotherapy is that certain chemotherapies sensitize tumors to IR induced DNA damage. However, the utility of non-targeted chemotherapies with IR is often limited by their dose limiting toxicity. Therefore, we hypothesize that tumor targeted radiosensitizing chemotherapies will increase IR induced anti-tumor immune responses. In Aim 1, we will determine if tumor targeted radiosensitization can increase the anti-tumor immune responses in combination with immune checkpoint blockade (CTLA-4 or PD-1 antibodies). We will use both syngeneic murine tumor models. Tumors will be grown in the bilateral flanks and radiosensitizing chemotherapies conjugated to tumor targeting peptides will be systemically administered. For IR, only the right flank tumor will be irradiated and the left flank shielded to measure the efficacy of IR induced systemic anti-tumor response. Immunotherapies blocking either CTLA-4 or PD-1 will then be tested in combination tumor-targeted radiosensitization. We will test if radiosensitizing chemotherapies modulate immune cells and increase T cell clonal diversity in blood and tumor tissues. Tumor xenograft response will be measured in paying close scrutiny to the effects on the left flank, non-irradiated tumor. In Aim 2, we will test the ability of IR and tumor targeted peptides conjugated to TLR7 agonists to increase anti-tumor responses. We will evaluate systemic immune activation through cytokine stimulation. Using bilateral tumor models we will also evaluate the efficacy of IR and targeted TLR7 agonists on tumor regression.

Preclinical development of ampakines for Chemo Brain

Host Campus: Irvine
Lead Investigator: Daniela Bota
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
This application focuses on the preclinical development of a pharmacological intervention for the prevention and treatment of chemotherapy-related cognitive impairments (Chemo Brain, CRCI). Millions are diagnosed with cancer every year and more than 60% now survive up to 20 years, unfortunately with severely diminished quality of life due to treatment-induced cognitive impairments. CRCI also influences the decisions of oncologists. Fear of CRCI constrains the doses and duration of chemotherapeutic regimens required for arresting tumors, with a diminished chance for cancer patients to achieve remission. Notably, research focused on therapeutic interventions for CRCI is still in its infancy. Clinical trials for medical treatments targeted at CRCI are currently unavailable. This proposal concentrates on preventing and reversing the cognitive impairments caused by cisplatin, a widely used ovarian cancer drug. This particular patient population develops CRCI consistently during and after cisplatin-based chemotherapy. The PI identified that the cognitive deficits caused by cisplatin seem to result from the loss of excitatory synapses and dendritic spines that anchor them, as well as from injury to adult and developing neurons. Furthermore, this neuronal toxicity derives from the decreased production of neurotrophic factors and especially brain-derived neurotrophic factor (BDNF). The PI now aims to identify safe and effective pharmacological treatments to ameliorate the cellular and synaptic damage and prevent or counteract the resulting learning and memory defects. BDNF- stimulated intracellular signaling is critical for neuronal survival and plasticity. In the hippocampus, BDNF regulates dendritic spine integrity. Our preliminary data shows that cisplatin severely decreases BDNF mRNA levels in primary hippocampal neuronal cultures, while treatment with ampakine (such as CX929) restored the cisplatin-induced BDNF down-regulation and ameliorated the dendritic damage caused by cisplatin treatment. Two specific aims are proposed: Aim 1. To determine if in vivo administration of the ampakine CX929 prevents synaptic loss and cell death of CA1 and CA3 hippocampal neurons provoked by a cisplatin regimen that recapitulates the cisplatin chemotherapy administered for ovarian cancer. Aim 2. To determine if in vivo administration of the ampakine CX929 prevents the learning and memory deficits provoked by a cisplatin regimen that recapitulates the clinical treatment for ovarian cancer.

Validation of the role of miR-483-5p in cholangiocarcinoma

Host Campus: Davis
Lead Investigator: Christopher Bowlus
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Cholangiocarcinoma (CCA) arises from the epithelial cells lining the bile ducts and carries a dismal prognosis. Primary sclerosing cholangitis (PSC), an autoimmune disease that targets the bile ducts, is the most significant risk factor for CCA with a 1% annual incidence of CCA in PSC patients. Early detection offers CCA patients some hope of cure. However, screening tests including serum CA19-9 and imaging are of limited value due to low sensitivity. In addition, when CCA is suspected, invasive tests have limited negative predictive values. Therefore, novel biomarkers for screening of high-risk groups such as PSC patients and for diagnosis are critically needed. Further, such biomarkers may potentially lead to novel therapeutic approaches. We have a long-standing interest in PSC and have recently begun to address the issue of CCA surveillance in this population. In a pilot study, we demonstrated that serum micro(mi) RNA expression patterns are distinct between CCA, PSC and healthy controls, providing a basis for the use of miRNAs as biomarkers for CCA surveillance. Importantly, miR-483-5p was expressed at greater levels in the serum of patients with CCA compared to patients with PSC without CCA, primary biliary cholangitis (another autoimmune disease of the bile ducts), and healthy controls, indicating that miR-483-5p is specific for CCA. However, it is unclear whether this microRNA is directly derived from the cancer cells or from the local responding cells or neighboring tissues. In addition, the molecular mechanism of action of miR-483-5p in tumorigenesis remains unknown. In this application, we will address these issues by: 1. testing if miR-483-5p is induced in CCA by comparing the expression of miR-483-5p in CCA tumor tissues versus adjacent non-tumor tissues and the expression and secretion of miR-483-5p in primary biliary epithelial cells versus biliary cancer cell lines; and 2. evaluating the effects of induced and repressed expression of miR-483-5p in CCA cell lines relative to cell motility, invasion and metastasis. This work will further support miR-483-5p as a novel biomarker for CCA surveillance; lay the groundwork for future investigations into the role of miRNA-483-5p in CCA; and determine the potential of a miRNA-based target for CCA.

Genetic signature of etheno DNA damage

Host Campus: Santa Cruz
Lead Investigator: Manel Camps
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Markers for exposure to chronic oxidative stress are needed to help estimate risk of cancer for tissues at risk of inflammation-induced cancer such as liver, esophagus or colon. Oxidative stress leads to lipid peroxidation, which in turn results in the generation of exocyclic adducts in DNA bases known as etheno adducts that are highly mutagenic. Here we propose the identification of a specific mutation signature for etheno DNA damage as a marker for exposure to chronic oxidative stress. We propose determining such signature by chemically inducing this damage in cells, sequencing the mitochondrial genome of treated cells by nanopore sequencing, and inferring an etheno-adduct specific signature using algorithms developed for analysis of cancer genomic databases. Mitochondria are selected as the target genome for several reasons: (a) a closed circle of DNA allows us to maximize the signal to noise ratio, (b) this organelle is highly susceptible to lipid peroxidation, (c) its DNA is abundant and redundant, facilitating DNA recovery and producing a spectrum that is less biased by positive selection than chromosomal DNA, and (d) studying mitochondrial genetic instability is of etiological value as it often plays a direct role in carcinogenesis. This project should fill in several gaps in our understanding of the connection between chronic exposure to oxidative damage and carcinogenesis This work will shed light into mechanisms of lipid peroxidation-induced carcinogenesis and lead to a better understanding of the sources of genetic instability in tumors. This information would be invaluable to link genetic data with epidemiological work linking infection and exposure to oxidizing agents and increased risk of cancer. In addition, this project has an important translational dimension as a biomarker for exposure to chronic oxidative stress .

Biologic Basis of Liver Cancer from Chronic Hepatitis B

Host Campus: Davis
Lead Investigator: Eric Chak
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Hepatocellular carcinoma (HCC) resulting from chronic hepatitis B (CHB) is one of the clearest examples of a cancer health disparity. According to recent Surveillance Epidemiology and End Results (SEER) data, Asian Americans continue to have highest incidence rate of HCC and HCC-related mortality compared to whites. The data also suggest that inequalities exist among different Asian ethnicities as Laotian/Hmong and Cambodian patients have worse HCC-related mortality when compared to other Asian ethnicities. Under an NCI-funded R21 grant entitled: "Biologic Basis of Disparity in Liver Cancer Survival Among Asian Americans," we are using next-generation sequencing (NGS) techniques to analyze viral and host factors which could explain these disparities in HCC-related mortality among Chinese, Vietnamese, and Laotian/Hmong patients with CHB. Viral factors such as B genotype and high viral loads have been associated with poor prognosis. Viral mutants including the A1762T/G1764A basal core promoter (BCP) mutations and Pre-S region deletions are also associated with poor HCC outcomes and may be the result of selection by host immune responses and activation of endogenous cytidine deaminase by pro-inflammatory cytokines (e.g., TNF-?) induced by NF?-B leading to G-to-A hypermutation. From this data, we will produce a blood expression profile that separates patients who are at "high risk" versus "low risk" for the development of HCC. However, our results need to be validated in a cohort of patients with HBV-related HCC. Specific Aim #1 would be to validate our initial findings on blood samples from patients diagnosed with HBV-related HCC. We performed a Cohort Discovery and approximately 60 patients with HBV-related HCC are currently within the UC Davis health system which should provide adequate samples for our assays. Specific Aim #2 will be to stain archived HCC tissue for HCC-associated proteins. Development of a risk profile which can be determined by a blood sample would be an important step towards personalized medicine in CHB management as "high risk" patients could be surveyed and monitored more closely than "low risk" patients.

The Histone H4K20me3 Mark as a Therapeutic Target

Host Campus: Los Angeles
Lead Investigator: Hilary Coller
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Histone modifications are being intensively investigated as tumor markers and targets for therapy. The histone H4 lysine 20 trimethyl (H4K20me3) mark is consistently downregulated in multiple types of tumors. Low levels of H4K20me3 are associated with poor prognosis for colon and breast cancer. However, whether H4K20me3 actively drives cancer progression, the mechanism by which it promotes tumorigenesis, and the most relevant methyltransferase, remain unknown. We propose to address these knowledge gaps. Our goal is to develop cancer therapy that will reintroduce H4K20me3 into patients and thereby limit tumor growth. We discovered that H4K20me3 is expressed at higher levels in quiescent than proliferating fibroblasts. Our ChIP-Seq data revealed that when fibroblasts enter quiescence, H4K20me3 is deposited at genes important for cell fate commitment. Surprisingly, in quiescent, but not proliferating fibroblasts, H4K20me3 is present not only as a repressive mark at the promoter but also in the gene body, and where it is associated with increased gene expression. Downregulation of a methyltransferase that generates H4K20me3, Suv4-20h2, results in increased cell proliferation and reduced chromatin compaction. Our preliminary data also show high levels of H4K20me3 in quiescent hair follicle stem cells and that these levels decline when hair follicles are actively cycling. We propose to investigate the genomewide pattern of H4K20me3 marks in quiescent mouse hair follicle stem cells, their proliferating progeny, hyperplastic hair follicles, and squamous cell carcinomas that arise when Ras is activated and p53 is inactivated in hair follicle stem cells. Further, to determine the functional role of the Suv4-20h2 enzyme in H4K20me3 deposition, we will monitor tumor develop and perform ChIP-Seq for H4K20me3 in mice with homozygous deletion of Suv4-20h2. Reduced levels of H4K20me3 in tumors represents an exciting opportunity for the development of new therapies that could be effective against multiple tumor types. We anticipate that our experiments will allow us to assess the functional role of H4K20me3 in a stepwise model of cancer progression. The findings will provide insight into the mechanisms through which H4K20me3 loss drives tumorigenesis and whether reactivation of Suv4-20h2 represents a promising approach to anti-cancer therapy.

Targeting Glycosidases Using Transition State Analogs

Host Campus: Davis
Lead Investigator: Sheila David
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
There is a rich history of the use of aza-sugars as tight-binding inhibitors of glycosidase enzymes and several have shown promise as potential pharmaceuticals. The efficacy of aza-sugar inhibitors is due to their mimicry of the oxacarbenium-ion like transition state (TS) that provides for favorable electrostatic interactions within the enzyme active sites. For example, pyrrolidine homonucleoside TS mimics of purine nucleoside phosphorylases (PNPs) have yielded extremely potent inhibitors that are presently in clinical trials as cancer chemotherapeutics. In addition, the mechanistically similar human enzyme 5-methylthioadenosine phosphorylase (MTAP) has also been suggested to be a potential cancer drug target due its alteration of S-adenosylmethionine levels in cells. Notably, similar azanucleotides incorporated within DNA are also tight-binding inhibitors of base excision repair (BER) glycosylases that have been exploited for use in mechanistic and structural studies. Targeting BER enzymes has also become an attractive new chemotherapeutic strategy and has promise in cancers that harbor other DNA repair defects. We propose to develop a generic strategy that will facilitate the development of new inhibitors for glycosidase enzymes by synthesis of a pyrrolidine nucleoside containing an appended alkyne. Using Cu-catalyzed alkyne-azide cycloaddition (“click”) reactions, the alkyne moiety will be further elaborated into a base-like substituent. Our hypothesis is that this will allow for the development of inhibitors that exhibit high specificity and affinity for a given glycosidase by engendering additional interactions near the active site. This approach will also make it possible to prepare a library of TS analogs that can be screened as inhibitors with various glycosidase enzymes. We will evaluate the use of this approach to prepare inhibitors for glycosidases that act upon nucleosides (PNP and MTAP). We also propose to synthesize an alkyne-substituted pyrrolidine 2’-deoxynucleoside phosphoramidite monomer that will be amenable to incorporation into DNA via solid phase DNA synthesis. In this case, the ability to prepare a library of inhibitors after DNA synthesis is particularly attractive since it will reduce the time required for synthesis. Screening methods will be developed using fluorescence-based enzyme assays to allow for high-throughput analysis of the “click” TS mimic libraries with various glycosidase enzymes.

Macropinocytosis as an energy source for prostate cancer

Host Campus: Irvine
Lead Investigator: Aimee Edinger
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Recent high-profile reports demonstrate that activated Ras drives macropinocytosis that allows pancreatic cancer cells to grow in low nutrient conditions. Through macropinocytosis, bulk extracellular fluid is engulfed via actin-dependent membrane ruffling that produces macropinosomes that are up to 2 um in diameter. Upon degradation in the lysosome, macropinocytosed proteins can supply amino acids for growth in poorly perfused areas of tumors. Inhibiting macropinocytosis slows the growth of Ras-driven xenografts without affecting the growth of tumors not exhibiting macropinocytosis. At present, macropinocytosis is believed to occur only in cancers with activating mutations in Ras. My lab has made the exciting and novel discovery that prostate cancers also use macropinocytosis to sustain their growth and survival in low nutrient conditions. As the nutrient sources for prostate cancer cells are poorly defined, our discovery that prostate cancer cells acquire nutrients by macropinocytosis is particularly significant. Targeting macropinocytosis could provide a novel therapeutic approach in castration resistant prostate cancer. New therapies are desperately needed for prostate cancer as patients invariably become resistant to even the newest androgen deprivation therapies, and cytotoxic chemotherapy extends life by less than 3 months. Several small molecules are available that inhibit macropinocytic flux, and these compounds might present a novels means to limit prostate cancer cell growth and survival alone or in combination with existing drugs. While my lab has previously studied nutrient transporter trafficking, macropinocytosis is a completely novel area of investigation for my group.

The role of LINC complexes in DNA repair and cancer

Host Campus: Davis
Lead Investigator: JoAnne Engebrecht
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
DNA repair pathways protect cells from the constant assault of DNA damage. While it is clear that failure to properly repair damage can result in mutations and ultimately cancer, the interactions, roles and communication between DNA repair pathways and the nuclear envelope have not been explored. The LINC complex (linker of nucleoskeleton and cytoskeleton) physically connects the nucleus to the cytoplasm and is essential for nuclear migration, cellular organization, and mechanotransduction. Mutations in LINC have been associated with colorectal, ovarian, breast, and lung cancers. It is unknown how LINC contributes to cancer, but LINC complexes have been implicated in the efficient repair of DNA damage. Our labs recently discovered that a novel LINC complex mediates DNA repair choice through linking the cytoskeleton with key repair proteins. Inactivation of LINC in both worms and human tissue culture cells results in sensitivity to cancer chemotherapeutic DNA crosslinking agents that is similar to members of the cancer-susceptibility syndrome, Fanconia Anemia. Sensitivity is rescued by inactivation of the non-homologous end joining (NHEJ) pathway, suggesting that the normal function of LINC complexes is to suppress error-prone NHEJ in favor of the more precise homologous recombination. LINC also recruits the Fanconia Anemia nuclease FAN1 to the nucleoplasm, suggesting that LINC influences repair by both inhibiting NHEJ and promoting processing of crosslinks by FAN1. Changes in FAN1 expression have been implicated in pancreatic and colorectal cancer. Here we propose to leverage this novel finding to: 1) Elucidate the molecular interactions between LINC, NHEJ, Fanconia Anemia components and FAN1 in the nucleoplasm. We will immuno-precipitate LINC from human tissue culture cells and determine whether components of NHEJ, Fanconia Anemia and/or FAN1 are associated. We will also identify novel DNA damage-induced interactions through mass spectrometry analyses. 2) We will determine whether depletion of LINC sensitizes cancer cells to crosslinking agents. We will use a variety of different cancer cell lines, including breast, pancreatic and colorectal. Together, these experiments will provide mechanistic understanding of the interactions between the cytoskeleton, the nuclear envelope, and DNA repair proteins and may lead to a novel approach for treating cancer.

Mechanisms of Chemotherapy Induced Peripheral Neuropathy

Host Campus: Santa Barbara
Lead Investigator: Stuart Feinstein
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Our understanding of the mechanisms of action of anti-cancer microtubule targeted agents (MTAs) is based almost completely upon either (i) in vitro biochemical experiments or (ii) experiments performed on cultured dividing cells treated with the drugs for short durations (hours to a couple of days). This is logical in terms of investigating their anti-cancer cell activities. However, chemotherapy-induced-peripheral neuropathy (CIPN) is a serious and common side effect of longer-term MTA treatment (weeks to months, such as in a clinical setting) that can be severe enough to be dose limiting, can require cessation of treatment and can even be life threatening. Since the affected peripheral nerves mediating severe pain are post-mitotic, the onset and progression of CIPN must result from non-mitotic effects of the MTAs. Different MTAs have different probabilities of inducing CIPN, likely because of their different mechanisms of action. However, since our understanding of MTA action is derived from analyses using either in vitro reactions or dividing cells in culture exposed to a MTA for short durations, our understanding MTA action on post-mitotic neurons for longer durations is negligible. Our current understanding does not consider the possibility of compensatory or regulatory mechanisms that come into play over longer, clinically meaningful durations. For example, recent work has demonstrated that the MTA eribulin, a microtubule depolymerizer in vitro and in short-term cell culture assays, leads to a dramatic increase in tubulin acetylation (a marker for stable MTs) in mouse sciatic nerves after two weeks of systemic administration. Here, we propose (i) to determine both the short-term and long-term effects of each of four FDA approved anti-cancer MTAs upon microtubule related components in human nociceptor nerve cells, the exact cell type that mediates CIPN and at the same time test the hypothesis that long-term and short-term effects of the drugs are distinct, and (ii) to take a more global view of CIPN by determining and analyzing the transcriptomes of short-term and long-term MTA treated human nociceptor nerve cells. This latter effort will lay the foundation for subsequent work by identifying and focusing upon transcripts whose abundance is altered by long-term MTA exposure.

Cancer systems biology analysis of malignant melanoma

Host Campus: Merced
Lead Investigator: Fabian Filipp
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
The epigenetic modifier EZH2 is in the center of a repressive complex controlling differentiation of normal cells. In cancer EZH2 has been implicated in silencing tumor suppressor genes. Its role in melanoma as well as target genes affected by EZH2 are poorly understood. We propose an integrated systems biology approach to analyze next generation sequencing data of melanoma cells to identify EZH2 target genes. Treatment with EZH2 inhibitor will be instrumental to determine molecular signatures involved in tumor suppression and immune recognition contributing to the aggressiveness of malignant melanoma.

S1P-driven Cell Growth in Lung Cancer of Smokers

Host Campus: Davis
Lead Investigator: Tzipora Goldkorn
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Sphingolipids are widely-implicated in respiratory illnesses and lung cancer. For example, ceramide, a sphingolipid linked to apoptosis, generates emphysema-like/COPD (chronic obstructive-pulmonary disease) phenotypes when upregulated in the lungs. Furthermore, neutral sphingomyelinase 2 (nSMase2), which hydrolyzes sphingomyelin to ceramide, becomes activated by cigarette smoke (CS) exposure and rapidly generates ceramide, which causes apoptosis. In contrast, sphingosine1-phosphate (S1P) is a sphingolipid associated with proliferative signaling and plasma S1P levels are elevated in lung cancer. Notably, lung cancer occurrence, such as Non-Small Cell Lung Cancer (NSCLC), is much higher in patients who have smoking-related COPD. However, it is unknown if S1P levels can predict lung cancer development from smoking-related COPD and if unique mechanism(s) for S1P production exist in smokers. Based on recent data, we hypothesize that human airway epithelial (HAE) cells with chronically-elevated nSMase2 expression and ceramide generation, as seen in smokers, are a novel source of S1P. nSMase2 is over-expressed in smokers which augments ceramide-induced apoptosis. Ceramide converted to Sphingosine becomes a substrate for Sphingosine Kinase 1 (SphK1) to produce S1P. SphK1-dependent S1P is then secreted to activate proliferative signaling and potentiate early lung cancer stages. To address our hypothesis we focus on the following specific aims: Aim 1: Demonstrate nSMase2, ceramide, SphK1, and S1P levels are up-regulated in lung tumors of patients who are smokers (compared to tumors of non-smokers) at different stages of NSCLC. Aim 2: Demonstrate in vivo (patient tumor xenograft model in NSG mice) that nSMase2 over-expression and augmented activity as well as SphK1's promote tumorigenesis and resistance to chemotherapy in lung tumors of smokers. This project completely differs from the PI's previous research goals and CRCC application as it does not focus on EGF receptor in lung cancer or ceramide's role in apoptosis, but focuses on: 1) translational research to validate nSMase2 and S1P as bona fide targets unique to smoking-related lung cancer, 2) molecular mechanism(s) of S1P-mediated cell proliferation and tumor growth in lung cancer, and 3) confirm the roles of nSMase2 and SphK1 in S1P production, targets amenable for therapeutic intervention in lung cancer. * Please note: original 3 aims (LOI-ABST) were consolidated into 2 aims due to space limits.

The role and molecular mechanism of PIR-1 in gene regulation

Host Campus: Riverside
Lead Investigator: Weifeng Gu
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
This project aims to investigate the function and mechanism of a highly conserved RNA polyphosphatase, PIR-1 (PIR1), in regulating genes and cell proliferation in DNA-damaged cells. Cancer cells usually exhibit loss of growth control with abnormal patterns of gene expression. A better understanding of how cell proliferation is controlled at gene expression level may provide novel and more effective targets for cancer diagnostics and therapeutics. RNA phosphatases usually play important roles in regulating RNA stability. Unlike its closest paralogs, mRNA capping enzymes, PIR1 belongs to a novel RNA polyphosphatase family which removes both beta and gamma phosphates from triphosphorylated RNAs (pppRNAs). The resulting RNA only has a 5' monophosphate and may be subject to decay by 5' to 3' exonucleases. Our preliminary results indicate that PIR1 is involved in two pathways regulating RNAs in C. elegans: 1) interacting with Dicer to silence viral transcripts with a 5' triphosphate group; 2) regulating thousands of genes in sperms and embryos using double-stranded pppRNA intermediates. Although the physiological substrates of human PIR1 (hPIR1) have not been clearly defined, studies have implicated hPIR1 in RNA splicing, consistent with its nuclear localization. The expression of hPIR1 is induced in a p53-dependent manner in cells treated with DNA-damaging chemicals, and the increased hPIR1 level may contribute to p53-induced cell cycle arrest in these cells. In addition, ectopic expression of hPIR1 inhibits cell proliferation, and repression of hPIR1 causes increased levels of cell proliferation. Our preliminary analysis suggested that hPIR1 may also interact with Dicer in HEK293T cells. Since PIR1 is highly conserved, we propose that hPIR1 may also regulate pppRNAs including nascent transcripts, and regulate cell proliferation in DNA-damaged cells. To understand how hPIR1 regulates cell proliferation, we will identify the RNAs targeted by hPIR1. Since hPIR1 may regulate multiple RNAs, normal mRNA-seq may fail to distinguish between direct targets and indirect ones. Here we propose to perform cross-linking immunoprecipitation (CLIP) coupled with high-throughput sequencing to systematically identify RNAs directly bound with hPIR1. And we will also investigate how hPIR1 modifies these targets to regulate cell proliferation in DNA-damage induced p53 pathway.

Nanoparticle capsules for combined release and hyperthermia

Host Campus: Merced
Lead Investigator: Linda Hirst
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
We propose to develop and test a potential new therapeutic system for tumor treatment using novel nanoparticle-based capsules recently developed in our lab. The capsules are hollow gold shells formed from closely packed nanoparticles. These capsules can be non-invasively optically triggered to release their contents while simultaneously heating their local environment (hyperthermia therapy). We recently developed a new mechanism to generate the nanoparticle-based microcapsules using gold NPs, assembled using liquid crystal ligands. The method is generalizable to different NP types and we have most recently produced gold-based capsules from 10nm particles. In this project we will develop this capsule technology for use as a multifunctional cancer therapy. The three key functions of the capsule, loading, triggered release and localized heating have been demonstrated in preliminary data. We aim to develop this novel capsule technology for applications in cancer nano-medicine using spherical and rod-like gold particles (for infra-red stimulation compatible with existing technologies) and outline a plan to test the capsules for triggered release and simultaneous local hyperthermia both in-vitro and in cells.

End-of-Life Care for Cancer Patients in the United States

Host Campus: Davis
Lead Investigator: Jeffrey Hoch
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
The United States offers government-financed health insurance for the elderly, allowing studies comparing care at the end-of-life for cancer patients. Previous research has shown that lung cancer patients use extensive end-of-life care. This amounts to millions of dollars being wasted to provide care that is neither clinically indicated nor desired by patients. Research discovering services that are more costly and less effective at producing what patients want are useful for policy makers, oncology clinicians and cancer patients. More research is needed to explore whether the quality of care for people at the end-of-life is improving through time, and if not, areas in which we can direct improvement efforts. With growing recognition of palliative care's place in oncology, it is vital to ascertain whether the knowledge of good end-of-life care is being translated into the practice of end-of-life care. In this proposed research, we will compare patterns of end-of-life care for elderly (>65 years of age) lung cancer patients who died of cancer during 1999-2001 vs 2009-2011. Lung cancer is an awful disease with a fearsome mortality rate. As such, high quality end-of-life care is of utmost importance. We will evaluate end-of-life care using the National Cancer Institute Surveillance, Epidemiology and End Results (SEER)–Medicare data, a linkage of patient records from the SEER cancer registries with their Medicare enrollment and claims files. The SEER registries collect demographic and clinical information for each patient including data for each occurrence of a primary incident cancer, date of diagnosis, follow-up vital status, and date and cause of death. The Medicare data, include claims for each beneficiary with fee-for-service coverage, with information about chemotherapy use and reduced emergency room (ER) visits and hospital use. We will estimate utilization rates per person-months adjusting for demographic differences. This research will provide evidence about the quality of end-of-life cancer care. Improvements in quality of care in the months before death will be evidenced by decreased rates of chemotherapy use and reduced ER and hospital use. In addition, we will assess quality of end-of-life care by sociodemographic factors to identify disparities.

Isolating a spliceosome complex target of anti-tumor drugs

Host Campus: Santa Cruz
Lead Investigator: Melissa Jurica
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Background: The SF3B complex is a core component of the spliceosome and directly involved in pre-mRNA splicing during human gene expression. Several SF3B components have been linked to human cancers. In particular, chronic lymphocytic leukemia and myelodysplastic syndrome patients exhibit a high frequency of specific point mutations in the SF3B1 protein. SF3B1 is also the molecular target of compounds that several pharmaceutical companies are developing as chemotherapeutics. We recently discovered that these compounds, which have very different structures, compete for the same interaction with SF3B1 to inhibit spliceosome assembly and splicing. Problem: The mechanism by which SF3B participates in splicing is currently unknown. We also do not understand where and how splicing inhibitors interact with SF3B1 to modulate its function. This information is key to understanding how SF3B1 mutations are involved in cancer and to improving the SF3B1 inhibitors as chemotherapies. Efforts to study SF3B structure / function have been stymied by several challenges. We recently overcame a key hurdle to obtain cDNA clones of all seven components and are now in the unique position to reconstitute the SF3B complex, which will allow us to finally address these questions. Investigators: The Jurica lab has extensive experience in studying macromolecular complex structure and function in the context of spliceosomes. We also are partnered with a top organic chemist, Dr. Arun Ghosh at Purdue University, who has generated a variety of inhibitor derivatives to probe SF3B1 function. Approach: We will take two approaches to isolating the entire SF3B complex: 1) We will co-express the seven SF3B proteins in a cell-free expression system, which will allow the proteins to fold and assemble with their complex partners. Preliminary data show that, individually, the cDNA are readily transcribed and translated in the system. In addition to structural analysis of purified complexes by electron microscopy, we will use them to investigate SF3B1 inhibitor interactions. 2) We will use HeLa cells engineered with a CRE/LOX recombination site to generate stable cell lines expressing tagged SF3B1 constructs. Preliminary data shows that active splicing extracts can be prepared from these cells, which we will use to test the effect of SF3B alterations on spliceosome assembly and splicing. Furthermore, we will use these cells to isolate endogenous SF3B complexes if the cell-free system proves intractable.

APC mutants activate a pre-cancer program

Host Campus: Davis
Lead Investigator: Kenneth Kaplan
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
The work to be proposed in the 2016 CRCC application represents a new direction for my lab. The focus is on the question of how normal cells are re-programmed prior to cancer onset in order to cope with the stresses caused by oncogene activation and aneuploidy. This question arose from our studies on chromosome instability in colorectal cancer, where we have shown that dominant, monoallelic mutations in adenomatous polyposis coli (APC) frequently found in human cancers, perturb microtubule dynamics and cause mitotic failures in intestinal crypts. The new research direction developed from recent findings in my lab, which show that perturbation of cytoskeletal dynamics by APC mutants activate a unique cell stress pathway. This pathway results in an increase in expression of a subset of heat shock proteins (Hsps) through activation of the Hsf1 transcription factor - a master regulator of the cell stress response required for the development of many cancers. Significantly, we found that normal intestinal crypt cells adjacent to cancer lesions in APC mutant mice also have elevated Hsp levels and activated Hsf1, a state of cell re-programming that anticipates the changes in nearby cancer cells. The idea that non-cancer cells adjacent to lesions exhibit signs of cancer re-programming is reminiscent of the cancer field effect (a.k.a., “field cancerization”), first described in 1953 by Slaughter and colleagues. The cancer field is thought to represent a pool of cells that can transition to cancer cells, but the nature of the initiating events or signaling pathways needed to establish the cancer field has remained unclear. We propose that the activation of Hsf1 is part of a “pre-malignant” pathway that contributes to the cancer field and creates a permissive cellular environment for cancer cell transformation. The CRCC proposal will identify the full array of transcriptional changes associated with the pre-malignant pathway both in cell culture and in intestinal crypts. This data will support one specific aim of a larger NIH-RO1 proposal on this new research focus.

Cancer Disparities in Young Adults with Medicaid Insurance

Host Campus: Davis
Lead Investigator: Theresa Keegan
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Cancer incidence in adolescent and young adults (AYAs: ages 15 to 39 years) is increasing and the leading cause of non-accidental deaths in this age group. While great strides in survival have occurred for younger and older cancer patients, less improvement has occurred among AYAs. Factors contributing to lesser progress in survival include poor access to health and supportive care, as AYAs have historically been the most highly uninsured in the USA. Nationally-representative studies have found that lacking insurance or having public health insurance at diagnosis or initial treatment were associated with diagnosis at an advanced stage, under-treatment and worse survival among AYAs with cancer. Our own work in California found an over 50% increased risk of cancer mortality among AYAs without private insurance. Prior to the Affordable Care Act (ACA), California extended Medicaid coverage to eligible uninsured patients after a cancer diagnosis. As a result, we were previously unable to distinguish those who were uninsured from those who were publicly insured at the time of diagnosis. As those who join Medicaid at diagnosis may miss out on cancer screening and other non-emergent care, it is important to identify patients uninsured at the time of diagnosis. Indeed, compared with being enrolled >6 months prior to diagnosis, Medicaid enrollment at diagnosis was associated with being diagnosed at a more advanced stage, fewer definitive operations and higher one-year mortality in older patients (mean age, 54 years) with selected cancers. However, no study has focused on continuous Medicaid enrollment in the highly uninsured AYA population or compared outcomes to AYAs with private insurance to estimate the potential gap in quality of care. Therefore, we propose to link Medicaid enrollment files to California Cancer Registry data for AYAs diagnosed with the twelve most common cancers during 2001-2013 and determine the impact of health insurance (uninsured, Medicaid insured prior to cancer diagnosis, Medicaid insured at diagnosis or privately insured) on stage at diagnosis, time to and type of treatment, and cancer-specific survival. With the expansion of Medicaid in 2014 as a source of health insurance in California, it is important to determine the effect of health insurance status on vulnerable subgroups of patients at risk for poor cancer outcomes as well as provide preliminary data for studies that assess health care utilization and cancer outcomes after implementation of the ACA.

Mutant p53 Reprogramming of the Colon Cancer Transcriptome

Host Campus: San Diego
Lead Investigator: Shannon Lauberth
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
The specific focus of the proposed study is to investigate the mechanisms by which chronic proinflammatory signaling by cytokines impacts the colon cancer gene landscape controlled by gain-of-function (GOF) p53 mutations, which are the most common lesions present in human cancers. Insight into this problem will advance our understanding of the broader paradigm of chronic inflammation-induced colon cancer. Through large-scale chromatin mapping, we identified a gain in mutant p53 binding to differential genomic regions in response to chronic TNF signaling. Once bound to these sites, we find that mutant p53 through cofactor recruitment establishes epigenetic changes linked to the potent induction of a subset of genes associated with proliferation, invasion, and metastasis. Building on these observations, my lab will test the key hypothesis that crosstalk between mutant p53 and TNF signaling pathways alters the GOF mutant p53 cistrome to create new opportunities for aberrant gene regulation. In an effort to provide insight into how inflammatory signaling molecules impact the temporal dynamics, nature, and strength of mutant p53-driven gene regulation, I have developed a novel “test tube” approach that consists of all isolated cellular components required to recreate gene control by common GOF p53 mutants on chromatin templates. This approach will provide significant insight into direct causal effects of tumor-promoting signaling molecules and changes in transcriptional activity with relevance to variability in GOF phenotypes associated with different p53 mutants. Pairing the mechanistic strengths of our “test tube” assays with genomic and molecular analyses in human colon cancer cell cultures stands to transform our understanding of the precise mechanisms by which the immune microenvironment empowers mutant p53 reprogramming of the cancer cell transcriptome.

Exploring a new source of genomic instability in cancer

Host Campus: San Francisco
Lead Investigator: Joachim Li
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Genomic instability is a key hallmark of cancer as it can facilitate oncogenesis, tumor progression, and therapeutic resistance. The sources of this genomic instability are poorly understood, however. We have identified a potential new source of genomic instability in budding yeast by showing that DNA re-replication arising from deregulation of replication initiation proteins can greatly induce genetic alterations such as gene amplifications and chromosome aneuploidy. Several findings by others raise the possibility that this deregulation may also contribute to genomic instability in human cancers: (1) overexpression of Cdc6 and/or Cdt1, two replication initiation proteins, have been reported in many cancers; (2) a key regulatory axis that is often overly active in cancers (E2F-Rb) controls the transcription of numerous replication initiation proteins, including Cdc6 and Cdt1; and (3) overexpression of Cdc6 or Cdt1 can induce tumors in certain mouse cancer models. Unfortunately, further investigation of this possibility has been stymied by lack of an assay that is sufficiently sensitive to detect re-replication in cancer cells. The problem is that re-replication is such a potent source of DNA damage that levels detectable by conventional assays are lethal. Thus, if re-replication does indeed contribute to genomic instabilty in some cancers, it must occur at cryptic levels that are currently undetectable. We propose to develop a more sensitive single-molecule assay for re-replication. In this assay, we will label DNA synthesis at two distinct periods within a single cell cycle using two fluorescently distinguishable nucleotide analogs. We will then use DNA molecular combing and established image analysis software to look for rare chromosome fragments that show overlapping incorporation of the two nucleotide analogs. Initial assay development will be performed in budding yeast, where we have the ability to conditionally induce re-replication over a wide range of levels and have extensive positive and negative re-replication controls. The assay will then be optimized for mammalian cells using published cell lines that tolerate modest overexpression of Cdc6 or Cdt1. Successful development of a more sensitive re-replication assay would provide the first opportunity to explore the prevalence and influence of re-replication in cancer cells.

Metabolic regulation of cancer cells of origin for SCC

Host Campus: Los Angeles
Lead Investigator: William Lowry
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Our preliminary data suggest that hair follicle stem cells (HFSCs) use a mode of metabolism more dependent on glycolysis and that this is distinct from their progeny. We hypothesize that this metabolic state could be key to the ability of these cells to both remain dormant for long periods and also undergo synchronized periods of proliferation at the start of each hair cycle. Furthermore, as HFSCs have been identified as the cell of origin for squamous cell carcinoma (SCC), we hypothesize that their unique metabolism is critical in the process by which they become transformed. Our preliminary findings indicate that HFSCs possess a unique metabolic profile that may be critical for their maintenance and for their response to oncogenic insults. Importantly, they also suggest the possibility that the "Warburg Effect ", in fact, results from expansion of an already glycolytic subpopulation: the HFSCs. Aim 1. Assess the metabolic evolution of SCC in vivo The vast majority of tumors show hallmarks of a more glycolytic state, relative to the tissue of origin. In the case of SCC, the tumor is initiated by HFSCs, which we now know show evidence of utilizing glycolysis for their maintenance. Using LC-MS/MS-based metabolomics, in situ enzyme activity assays, and gene expression analyses to profile the cells that initiate tumors and through the subsequent stages of tumorigenesis, we will address the important question: are tumors glycolytic because the cell that gives rise to them is glycolytic? Aim 2. Define the functional role of metabolic states on tissue homeostasis and tumor initiation Our ability to specifically delete genes in particular cell types within the follicle in an inducible manner will be exploited to identify key metabolic nodes controlling both adult stem cell homeostasis and tumor initiation. Our preliminary studies have identified a key metabolic node that may play a critical role in the unique metabolism of HFSCs: lactate dehydrogenase (Ldh). We will probe the role of Ldh in particular and glycolysis in general through genetic loss of function in adult HFSCs to determine the role of glycolysis in homeostasis and tumor initiation.

Mechanisms of Rhabdomyosarcoma Development and Progression

Host Campus: Berkeley
Lead Investigator: Kunxin Luo
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
I am proposing a pilot study to initiate a new research project with the long-term goal to determine the molecular pathway that drives the development and progression of Rhabdomyosarcoma. Rhabdomyosarcoma or RMS, derived from myogenic precursor cells, is the most common pediatric soft tissue sarcoma, accounting for 5-10% of all pediatric malignancies and are a leading cause of cancer death in children with few therapeutic options. Clinically, RMS is divided into two major subtypes: embryonal RMS (ERMS: 60% of all cases) and alveolar RMS. While most alveolar cases are associated with a specific chromosome translocation event, the oncogenic drivers in the more common ERMS are poorly defined. Recent studies have shown that ERMS tissues display an elevated expression of YAP, the transcription effector of the Hippo pathway, and that high YAP activity promotes transformation of myogenic progenitor cells to ERMS. However, none of the upstream Hippo components have been altered in ERMS, suggesting that YAP expression and activity must be upregulated by a yet-to-be-identified regulator. We recently identified SnoN as a novel upstream regulator of YAP in mammary epithelial cells. Interestingly, SnoN was originally cloned from the RMS tissues, suggesting an intriguing possibility that SnoN may function upstream of YAP to promote RMS. In this pilot project, we will test the hypothesis that high levels of SnoN in ERMS enhance YAP activation and expression to promote ERMS development and progression using in vivo xenograft mouse models in combination with cultured human ERMS cell lines. Two specific aims are designed: 1) Compare the expression pattern of SnoN in ERMS tissues and cell lines with that in normal muscle tissues and cells and correlate that with the pattern of YAP expression and activation; 2) Examine the effect of reducing SnoN in ERMS cells on their transforming activity in vitro and on tumor growth in vivo. The effect of SnoN knockdown on YAP expression and activation will also be assessed. Finally we will introduce WT and a constitutively active YAP back to ERMS cells with the SnoN knockdown and ask whether YAP overexpression can rescue defects in transformation caused by SnoN deletion. The results from these preliminary experiments will form the foundation for a long-term grant to be submitted to a major funding agency.

MicroRNA-mediated differentiation of high-grade glioma

Host Campus: San Francisco
Lead Investigator: Anders Persson
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Glioma is the most common primary malignant brain tumor, with high-grade glioblastoma (GBM) among the most aggressive cancers in children and adults. Despite aggressive surgery, radiotherapy, and treatment with the alkylating agent temozolomide, the prognosis for GBM patients is dismal with an average survival of 12-15 months. Standard of care treatment successfully eliminates the majority of GBM cells in individual tumors, but fails to target minor cell populations displaying neural stem cell (NSC) properties. Transcriptomal subgroups of classical and mesenchymal GBMs show a NSC signature, including expressing of the transcription factor SOX9, and frequently express cancer-causing genes that act through the mitogen-activated protein kinase (MAPK) pathway. Oncogenic activation of the MAPK pathway blocks neurogenesis of NSCs and initiates GBM formation. Is it possible that restoration of down-stream effectors of the MAPK signaling pathway can turn tumor cells into neurons and reduce GBM aggressiveness? We hypothesize that normal neurogenesis is hijacked to drive tumor growth in SOX9-expressing GBMs, and that reactivating neurogenesis can reduce tumor growth and sensitize tumors to therapy. We propose that the neuronal determinant microRNA-124 (miR-124), a small non-coding RNA, is a critical down-stream effector of oncogenic MAPK activation, and when reinstated, will restore neurogenesis and reduce GBM aggressiveness. To address the hypothesis, we have generated novel reagents to test whether miR-124 overexpression reactivates neurogenesis to block initiation, progression, and resistance to radiotherapy and TMZ treatment in GBMs. In murine and patient-derived primary GBMs, we will in aim 1 we will clarify whether MAPK inhibition or miR-124 overexpression regulate SOX9 expression, stemness, and neuronal differentiation in tumorsphere cultures. We will also perform miRNA profiling following MAPK inhibition. We will then in aim 2 test whether MAPK inhibition or miRNA overexpression DNA response, DNA repair, proliferation, apoptosis, and differentiation in three human GBM tumorsphere cultures. Finally, we will test whether MAPK inhibition or miRNA-124 overexpression sensitize tumor cells to radiotherapy or TMZ treatment in GBM xenografts. Our work will manifest the MAPK-miR-124 axis as a driver of aggressiveness in SOX9-expressing GBMs, and when reactivated will turn tumor cells into neurons, ultimately improving the overall outcome in these GBM patients.

Harnessing the immune system to eliminate glioma stem cells

Host Campus: San Francisco
Lead Investigator: Claudia Petritsch
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Stem-like tumor propagating cells (TPCs) in glioblastoma utilize a polo-like-kinase 1 (Plk1)-controlled polarity checkpoint to divide asymmetrically to self-renew and generate heterogeneous tumor cells. TPCs are sensitive to Plk1 inhibition but exhibit intrinsic resistance not only to standard therapy, consisting of radiation and temozolomide, but also to small molecule inhibitors of MAPK signaling. MAPK signaling is frequently upregulated in glioblastoma in part due to a mutation in BRAF kinase, BRAFV600E. The programmed death-ligand 1 PD-L1, a negative regulator of T cell proliferation, is expressed on the surface of TPCs, which is thought to mediate immune suppression. Durable clinical anti-tumor responses are therefore expected from emerging immune therapies, including anti-PD-L1 antibody therapy, in combination with MAPK pathway inhibitors. To maximize the efficacy of novel immune therapies, the responses of TPCs to immune checkpoint inhibition needs to be elucidated. We propose that by combining anti-PD-L1 antibodies with MAPK pathway and Plk1 inhibitors in tumors with elevated MAPK signaling, the immune suppressive activity of TPCs will be reduced and tumor growth will be inhibited more effectively than with small molecule inhibitor therapy alone. While we have worked on BRAFV600E glioblastoma for several years, the study of the immune system interactions with TPCs and tumor cells are novel to the Petritsch lab. Using our novel syngeneic mouse models of glioblastoma, we will assess the effects of immune checkpoint inhibitor inhibition (ICI) on TPC, non-TPC tumor cells as well as tumor-resident immune cells alone and in combination with standard and small molecule inhibitors (MEKi, Plk1i) (aim 1). Mechanisms of resistance will be explored by single cell RNA sequencing analyses of the TPCs, which survive ICI therapy in mouse models (aim2). Upon completion of this aim, we will have determined if inhibiting immune checkpoint inhibitors such as PD-L1 and CTLA-4 in combination with small molecule inhibitors block tumor growth more effectively than either regimen alone. The data from the single cell analyses study will determine whether resistance to occurs due to transcriptional upregulation of alternative checkpoint inhibitors and other pathways, which prevent MAPK addiction. The studies combined will allow us to develop better anti-gliobastoma treatment regimens.

Effects of Microvasculature ECM on Glioblastoma Infiltration

Host Campus: Los Angeles
Lead Investigator: Stephanie Seidlits
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $54,929

Abstract:
Glioblastoma multiforme (GBM) is an extremely lethal cancer originating in the brain. A major reason for GBM aggression is the infiltration of the primary tumor throughout the brain, preventing complete surgical resection. In particular, GBM cells migrate in close association with tumor microvasculature. GBM infiltration strongly depends on biochemical and physical aspects of its microenvironment. Compared to healthy brain, GBM tumors are ~20x stiffer with increased extracellular matrix (ECM) deposition. For example, changes in the distribution of hyaluronic acid (HA) – a non-sulfated glycosaminoglycan that constitutes the majority of the ECM in the healthy CNS – are reported to increase drug resistance, proliferation and migration of GBM cells. To recapitulate this microenvironment in an experimentally controlled context, the Seidlits laboratory has developed ex vivo platforms that support physiologically relevant, 3D cultures of patient-derived GBM cells. These HA-based hydrogel platforms can be modularly constructed for independent control of microenvironmental properties – stiffness, density of HA and integrin-binding sites derived from native ECM proteins, and permeability. Although previous culture systems have been developed to mimic singular aspects of the complex GBM microenvironment, the proposed platform will enable study of the combinatorial effects of multiple pathological features. The Kornblum laboratory has fully characterized gene expression profiles in cells isolated clinical GBM tumors and their associated microvasculature. Using this data, they have constructed a virtual “interactome” to identify a short list of ECM proteins expressed by the vasculature that likely interact with migrating GBM cells. Aim 1 will evaluate the effects of integrin-binding peptides derived from ECM proteins on this list – SIBLINGs, tenascin-C and vitronectin – and matrix stiffness – hydrogels matching both healthy brain and GBM tumors – on proliferation and migration of GBM cells using 3D culture platforms developed by the Seidlits laboratory. Aim 2 will expand the capabilities of these platforms to present gradients of integrin-binding peptides and substrate stiffness, which have been previously indicated to promote directional migration of multiple cells types, in the direction of increasing peptide concentrations. In sum, we aim to quantitatively characterize the effects of the local microvasculature on GBM cell migration using an engineered, ex vivo model of the tumor microenvironment.

Wnt Regulation of Metabolic Heterogeneity in Cancer

Host Campus: Irvine
Lead Investigator: Marian L. Waterman
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Our recent discoveries have led us to link oncogenic Wnt signaling directly to glycolysis and Warburg metabolism. While this discovery connects to the well-known cancer hallmark of metabolic reprogramming - a cell-intrinsic feature important for anabolic support of cancer cell proliferation - we have also connected it to cell-extrinsic, environmental effects. We have shown that the Wnt-driven glycolysis activity of colon cancer cells creates a highly angiogenic tumor environment and that it promotes a Turing-like pattern of metabolism throughout the tumor – a form of non-genetic tumor heterogeneity not readily detectable by "omics" analyses. The pattern was discovered by probing for markers of metabolism in xenograft tumors and it is evident as a self-organizing spatial pattern of glycolysis wherein clusters of glycolytic cells are arranged in a spotted array. To probe the mechanism of this pattern, we developed a reaction-diffusion-based mathematical model that incorporates Wnt signaling, angiogenesis and Warburg metabolism. The model recreates the spotted array in 3D and makes several important predictions. In this application, we seek funding to test two predictions for validation. Aim I: test that canonical and non-canonical WNT signaling influences the pattern of metabolic heterogeneity. Aim II: test that metabolic heterogeneity derives from metabolic symbiosis. We propose genetic modification of colon cancer cells (CRISPR knockout, lentiviral transduction) for xenograft and orthotopic tumor analysis. Metabolic, genetic and imaging analyses will evaluate tumor heterogeneity, metabolic patterning and progression. The experiments will be a rigorous validation test of the mathematical model - an essential step for preparation of a competitive grant application to develop a systems biology level of understanding of oncogenic Wnt signaling and cancer metabolism, not just in mouse models, but in primary human cancer. The overarching goal is to explore new ideas for therapy that target weaknesses in this system. This study is a new direction in translational science for the investigator and has not been formally supported by peer reviewed funding.

Induced formation of human hepatocellular carcinoma in mice

Host Campus: San Francisco
Lead Investigator: Holger Willenbring
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
We propose to generate human hepatocellular carcinoma (HCC) from scratch to facilitate future studies aimed at identification of new drugs and biomarkers for early detection. HCC is a major clinical challenge because of its refractoriness to available therapies and increasing incidence. We hypothesize that much-needed breakthroughs in HCC therapy will require a humanized animal model because conventional animal models fail to replicate the complex genetics and biology of human HCC. To generate a faithful mouse model of human HCC formation, we will use mice highly repopulated with primary human hepatocytes. Specifically, using serial transplantation we have generated mice repopulated with human hepatocytes with critically short telomeres, a hallmark of chronic liver diseases and risk factor for HCC formation. To induce malignant transformation of these vulnerable human hepatocytes, we will replicate driver mutations identified in HCCs in patients, i.e., a telomerase-activating promoter mutation found in the majority of HCCs and disruption of CDKN2A, which regulates the critical tumor suppressors TP53 and RB. For this, we have developed a system for gene editing in human hepatocytes in vivo using CRISPR/Cas9 technology and human hepatocyte-targeted adenoassociated viral (AAV) vectors. Because each mouse harbors 10-50 million human hepatocytes, of which 50% are transduced by the AAV vectors after intravenous injection, we expect to generate in each mouse thousands of human hepatocytes that carry these carcinogenic mutations. To further model repeated hepatocyte turnover characteristic for chronic liver injury, we will isolate the mutated human hepatocytes and serially transplant them into our liver repopulation mice. We expect that combining HCC driver mutations with liver repopulation-induced replication stress, particularly in the context of genomic instability caused by telomere erosion, will lead to formation of dysplastic nodules and subsequently HCCs. The proposed research will define the carcinogenicity of telomere erosion and telomerase activation in human hepatocytes and may yield a mouse model that, by replicating the critical stages of human HCC formation, will shed light on the cell and molecular biology of this lethal cancer and facilitate screens for new anti-HCC drugs and biomarkers for detection at an early resectable stage.

Metabolic changes in response to DNA damage

Host Campus: Irvine
Lead Investigator: Kyoko Yokomori
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
DNA damage response (DDR)/repair are critical cellular processes for the maintenance of genome integrity. DDR dysregulation is intimately linked to cancer development, while at the same time offering therapeutic opportunities. Mutations of DDR genes, such as BRCA1 and ATM, increase cancer incidence, while genotoxic agents (e.g., cisplatin) and inhibitors of DDR such as PARP inhibitors are found to be effective for the treatment of certain types of cancers. Thus, better understanding of the mechanisms and regulation of DDR in human cells is critical for understanding cancer etiology and for the development of new/improved agents. The immediate early response to DNA damage involving damage recognition and repair pathway choice has been an area of intense research. However, the later steps of DDR (i.e., recovery and the long-term consequences of DNA damage) are not well understood. Increasing evidence indicates that the cellular energy state influences epigenetic states of chromatin, affecting various nuclear functions including DNA repair and gene regulation. There are distinct metabolic changes associated with cancers, including high rates of aerobic glycolysis (‘‘Warburg effect’’). We obtained evidence that DNA damage shifts the balance of energy metabolism between glycolysis and oxidative phosphorylation in a PARP-dependent manner, revealing the intimate relationship between the two processes and the critical role of PARP signaling. Correlating with this change, nuclear-wide histone acetylation changes occur. Based on these data, we plan to investigate the relationship between PARP signaling and metabolism, chromatin regulation, and DDR in human normal and cancer cells using high-resolution live fluorescence imaging techniques, and interrogate the consequence of damage-induced epigenetic changes in gene regulation using integrative high-throughput analysis of chromatin immunoprecipitation and sequencing (ChIP-seq) and RNA-seq. The goal of this project is to decipher how DDR influences metabolism to affect cell survival and genome stability. The outcome of this project may contribute significantly to more effective usage of PARP inhibitors and developing new strategies for cancer therapy.

Mitomycin C-inspired selective targeting of solid tumors

Host Campus: Santa Barbara
Lead Investigator: Liming Zhang
Start Date: 1/1/2017 *End Date*: 12/31/2017 *Amount*: $55,000

Abstract:
Mitomycin C is a potent anticancer drug but has had limited usage clinically because of its high off-target toxicity. Due to its complex and sensitive structure, efforts to improve it by its synthetic modifications have failed to deliver any clinically improved alternative. In this proposal a new class of bioreductive chemotherapeutics, i.e., nitropyrroloindoles, that is relevant to mitomycin C and inspired by its mechanism of bioreduction-activated lethal DNA double alkylation is designed and will be synthesized. The design employs a modified alkylation mechanism that has been validated by the preliminary study and incorporates an oxygen-sensitive bioreduction of nitroarene as the mechanism of activation, which permits selective targeting of hypoxic areas of solid tumors. These novel design features are facilitated by our prior established Pt catalysis, which offers a facile synthesis of the design molecules. The objective of this project is a) to synthesize the various designed nitropyrroloindoles for selective targeting of hypoxic regions of solid tumors and b) to discover potent lead compounds and generate convincing bioassay data via collaboration with Prof. Reich in order to secure a NIH grant for the pursuit of developing clinically viable anticancer agents. The successful implementation of this project would open new venue for developing selective and potent anticancer agents.