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Defining the irregular ER stress response of myeloma cells

Host Campus: Santa Barbara
Lead Investigator: Diego Acosta-Alvear
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Maintaining homeostasis is vital for cells. Cells have specialized mechanisms—stress responses—that detect internal imbalances and respond to them to restore homeostasis. However, if restoring homeostasis is impossible, these mechanisms induce cell death instead, thus removing injured cells to protect the organism. The aberrant genetic makeup of cancer cells predisposes them to chronic stress, as they show dysregulated gene expression and often produce defective or overactive proteins. To cope with chronic stress, some cancer cells alter their stress responses, becoming addicted to compensatory cell functions that enable tumor progression. Multiple myeloma (MM), a cancer of plasma cells, demonstrates this notion: in MM cells, chronic endoplasmic reticulum (ER) stress is offset by the proteasome, a key component of the protein homeostasis network. This renders MM hypersensitive to proteasome inhibitors, which have revolutionized MM treatment. Unfortunately, relapse is inevitable and MM remains incurable. Fulfilling the unmet need of new targeted therapies for MM requires the dentification of resistance mechanisms. We have shown that MM cells overcome proteasome inhibition through upregulation of autophagy components, suggesting an adaptive switch in protein turnover mechanisms (Acosta-Alvear, et al. 2015). Here we propose to define the mechanisms by which MM cells modify their unfolded protein response (UPR)—the ER stress response—to their advantage. We found that the UPR is enhanced in MM cells, allowing them to survive ER stress that kills other cells. Moreover, inhibiting the UPR hampers MM cell growth/division. These results suggest that MM cells rely on the UPR for survival. We will address the question of how MM cells respond and adapt to genetic or pharmacologic perturbations of the UPR in two specific aims. In aim 1, we will define the specific UPR components that confer a survival advantage to MM cells. In aim 2, we will follow up on our recent finding that blocking a single UPR component—the ER stress sensor kinase/RNase IRE1—protects MM cells from the harmful effects of inhibiting the G2 cell cycle-checkpoint kinase PKMYT1. Our work has the potential to reveal elusive genetic vulnerabilities that arise upon targeted intervention of the UPR in MM cells, exposing Achilles' heels that could be exploited for designing of new therapies for MM.

Patterns of Care and Outcomes in AYAs with Germ Cell Tumors

Host Campus: Davis
Lead Investigator: Elysia Alvarez
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Germ cell tumors (GCT) are the third most common cancer in adolescent and young adult (AYA: 15-39 years) patients and its incidence is increasing. Survival has increased significantly over the past three decades; however, disparities by stage of disease, age and sociodemographic factors remain. AYA patients with GCTs do not receive uniform care, with providers varying from pediatric oncologists, medical oncologists, urologists, to gynecologists. They also may be treated in community hospitals or specialized cancer centers (SCC: Children's Oncology Group and National Cancer Institute-designated centers). Treatment at SCCs or by pediatric oncologists has been found to improve outcomes in AYAs with certain cancers (i.e. acute lymphoblastic leukemia, Ewing sarcoma), possibly due to clinical trial enrollment and standardized care approaches, but these associations have not been assessed for AYA patients with GCTs. Most of what is known has been obtained through clinical trials, which while important, does not provide a complete "real world" picture of treatment, location of care and survival outcomes in this patient population. Therefore, we propose to undertake a comprehensive, population-based assessment using the California Cancer Registry (CCR) linked with statewide hospitalization data. This database captures information on nearly all patients with GCT in California—allowing us to determine patterns of care for AYA patients with GCTs. We aim to identify the treatment regimens administered and determine differences by location of cancer treatment and treating physician specialty; and examine the impact of guideline concordant care, location of care and treating physician specialty on survival. We hypothesize that guideline concordant treatment will differ by the treating specialty, especially in older AYAs, and that survival in patients with later stage disease will be superior if these patients are treated at SCCs and/or with guideline concordant care. We will accomplish these aims through the use of novel methods to abstract chemotherapy regimens (protocol, specific drugs), provider subspecialty (urology, oncology etc.) and surgical details (biopsy date, resection date, surgical margins) from text fields in the CCR. Findings from this study will identify potential areas for intervention that can improve survival outcomes in AYA patients with GCTs.

Multiplexed in vivo imaging of the tumor immuno-environment

Host Campus: San Francisco
Lead Investigator: Moshiur Anwar
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $73,750

Abstract:
A first-in-class, in vivo wireless biopsy consisting of an implantable miniaturized sensor of real- time responses within the tumor itself would provide a ground-breaking means to guide optimal, personalized patient care. To accomplish this, we will design a network of millimeter scale sensor "motes" relaying real-time tissue and immune response from directly within tissue.

Recent innovations in wireless power and data transfer and unprecedented integration of high- speed circuits with photonics will unlock an entirely new paradigm of real-time response assessment. In Aim 1, we will build a mm^3 chip-scale fluorescence-imaging mote, allowing in vivo fluorescence imaging of the tumor microenvironment from directly within the tumor, non-invasively and continuously. We demonstrate delivery of power to a deeply implanted micro-laser diode and imaging of fluorescently labeled cells.

In Aim 2, we utilize our images to achieve real-time imaging of the dynamic immune response in a mouse tumor model, providing an heretofore unobtainable view into the evolving tumor microenvironment. Using an immune component model of melanoma, we dynamically image surrogates of immune response: CD8+ T cells. Metrics of success are measured by linking in vivo imaging to tissue slices from the excised tumor.

Implementation of these sensors will enable dynamic readouts of each patient's tumor composition and response to therapy. This will facilitate customized application of novel therapeutics and immunotherapy tailored to the individual patient's tumor biology. Future efforts based on this preliminary data will seek to measure strategies to convert a "cold" tumor to a "hot" one by using various immune stimulating agents, such as radiotherapy in hard to treat disease, such as pancreas cancer. This project represents a fundamentally new direction in research for my laboratory and is not currently funded by any source.

Targeting Decreased Lysosome pH to Limit Cancer Progression

Host Campus: San Francisco
Lead Investigator: Diane Barber
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Cancer cells rely on an altered metabolism to generate biomass to fuel metastasis and rapid cell proliferation. One goal of current cancer research is to therapeutically correct altered metabolism to limit disease progression. Most efforts toward this goal focus on targeting intermediary metabolic pathways, including glycolysis, oxidative phosphorylation, and fatty acid catabolism. Our proposal tests an alternative idea of targeting dysregulated lysosome pH (pHlys) as a strategy to limit biomass production and proliferation. Lysosomes are catabolic hubs and in normal cells pHlys is ~ 5.0 for the activity of lumenal acid-activated proteases. In cancer cells pHlys is thought to be lower compared with normal cells, which we confirmed in breast and pancreatic cancer cells. A lower pHlys increases catabolism of macromolecules to increase biomass. We are applying for CRCC funding to begin testing the feasibility of therapeutically restoring pHlys to normal values to limit tumorigenic behaviors. However, resolving the feasibility of targeting pHlys for cancer therapeutics is currently constrained by two limitations. First, we lack tools to selectively measure pHlys, with current studies relying on phagocytosis of pH-sensitive dyes that accumulate in multiple intracellular compartments. Second, beyond the V-ATPase, which localizes in multiple intracellular compartments, lysosome-specific proteins regulating pHlys remain unknown but could be therapeutic targets. To resolve the first limitation we generated and validated a new genetically encoded pHlys biosensor (pHLARE) that localizes predominantly in lysosomes and was used to confirm that cancer cells of different tissue origins have a pHlys ~ 4.3. To resolve the second limitation we used pHLARE in a genetic screen with CRISPR/Cas9 silencing of lysosome-specific proteins and found that genetic silencing of ClC-7, a Cl-H transporter, significantly increases pHlys and hence could be a feasible therapeutic target. However, to validate feasibility requires confirming our hypothesis that correcting lower pHlys in cancer cells will limit tumorigenic properties. We will test this hypothesis by increasing pHlys in cancer cells with silencing ClC-7 and determine effects on proliferation (Aim 1), invasion (Aim 2), and oncogenic transformation (Aim 3).

Novel Links Between Wnt Signaling, Centrosomes, and Cancer

Host Campus: Irvine
Lead Investigator: Lee Bardwell
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Normal patterns of Wnt signaling are necessary for tissue development and maintenance, while hyperactive Wnt signaling is implicated in many cancers, especially colon cancer. Indeed, loss of the Wnt pathway negative regulator adenomatous polyposis coli (APC) is typically an early step in the progresson of colon cancers. APC loss results in the stabilization of beta-catenin, which then forms active heterodimers with LEF/TCF (lymphoid enhancer factor/T-cell factor) transcription factors. LEF/TCF target genes promote proliferation, migration, Waarburg-type metabolism, and survival, all of which contribute to malignancy.

Our preliminary experiments have uncovered intriguing new connections between the Wnt signaling pathway and the centrosome, the major organizer of the microtubule cytoskeleton in mammalian cells. Specifically, we have found that LEF/TCF transcription factors localize to the centrosome, where they interact with the centrosomal scaffold protein CEP152, and with the protein kinase PLK4. Futhermore, our preliminary studies indicate that that PLK4 phosphorylates the TCF1 protein and regulates the expression of LEF/TCF target genes. The centrosome is critical for the maintenance of genome integrity, and the PLK4 protein kinase is the master regulator of centrosome duplication, whose over- or under-expression causes tumor-promoting chromosomal instability. Thus, our findings suggest potential new connections between Wnt signaling and the maintenance of genome stability.

We propose a series of experiments to determine the mechanism and functional consequences of the centrosomal localization of LEF/TCF transcription factors, and of the phosphoregulation of LEF/TCF factors by PLK4.

We will determine the domain of CEP152 that binds to LEF/TCF transcriptional factors and the functional consequences of this interaction. In addition, we will map the site(s) of PLK4-mediated phosphorylation of TCF transcription factors, and investigate the functional consequence of this phosphorylation

The potential impact of the studies proposed herein is considerable. Our research could reveal new therapeutic opportunities for targeting the Wnt pathway, unexpected new connections between Wnt signaling and the maintenance of genome stability, and a novel mechanism by which the centrosome may communicate with the nucleus to regulate gene expression.

Mapping translocations induced by defective R loop removal

Host Campus: Davis
Lead Investigator: Jacqueline Barlow
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Recurrent DNA translocations characterize blood cell cancers, and are frequently products of aberrant repair of programmed DNA double-strand breaks (DSBs). Defining the precise molecular mechanisms governing DSB generation and repair is critical to revealing how lymphoid cancers arise. The majority of cancers involving antibody-producing B cells arise during class switch recombination (CSR), a programmed DNA repair event at the immunoglobulin heavy chain (IgH) locus. CSR is initiated by DSBs generated by the enzyme AID, whose recruitment to IgH requires transcription. AID-dependent DSB formation strongly correlates with the appearance of R loops—three-stranded nucleotide structures where newly transcribed RNA re-anneals to template DNA. Though R loops were observed at the IgH locus over 20 years ago, their role in CSR remains undefined. To investigate the role R loops play in CSR, we generated mice deficient for two enzymes promoting R loop dissolution, the helicase Senataxin (SETX-/-) and the nuclease RNase H2 (RNH2Bf/f). We find that SETX-/- RNH2Bf/f B cells have increased R loop formation. Over 10% of double-deficient cells accumulate unrepaired DNA breaks and translocations at IgH, while single mutants show modest IgH instability. All 4 genotypes are proficient for CSR, thus DSB repair is largely intact. These results show that Setx and RNase H2 act independently to promote genome stability and suppress aberrant DNA repair during CSR. To test our hypothesis that the presence or absence of R loops affects repair pathway choice, we propose to map genomic loci translocated to the IgH locus using High-Throughput Genome-wide Translocation Sequencing (HTGTS) in WT, SETX-/-, RNH2Bf/f, and SETX-/- RNH2Bf/f B cells undergoing CSR. Importantly, we will use long-read (150-300 bp) paired-end sequencing to capture DNA from IgH and partner loci and sequence overlapping the junction site. HTGTS maps will define the location and frequency of IgH translocation partners found in Setx, RNase H2 and double-deficient cells. Further, we will use translocation junction sequences to define the extent of homology found at the breakpoints, and infer the DSB repair pathways promoting translocation formation. Defining the precise mechanisms underlying R loop-associated translocation formation will provide novel molecular targets for cancer prevention and therapeutic intervention.

High-throughput analysis of DNA Repair Cancer Variants

Host Campus: Davis
Lead Investigator: Sheila David
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Variants of unknown significance (VUS) in cancer-causing genes are a glaring blind spot in genomic approaches to clinical cancer diagnosis. As more patients and clinicians gain access to both somatic and germline DNA sequencing, the utility of the sequencing information is hampered by the lack of definitive functional assessment of variants in cancer-associated genes such as those in DNA repair genes. Biallelic inherited mutations in the DNA base excision repair (BER) gene MUTYH lead to MUTYH-associated polyposis (MAP), a colorectal cancer syndrome, and less commonly, other forms of cancer. MUTYH prevents G:C to T:A mutations caused by the common base oxidation product, 8-oxoguanine (OG) by removing mis-placed As from OG:A mismatches. Reduced activity of MUTYH leads to somatic mutations in the tumor suppressor APC and the oncogene KRAS. Clinical reporting databases have grown to include over three hundred unique germline MUTYH variants, the vast majority VUS. It is unclear whether the large number of uncharacterized MUTYH variants are silent polymorphisms or deficient in DNA repair. We propose to determine the cancer-causing potential of a large number of MUTYH missense and nonsense variants in human cells using an innovative multi-faceted approach that leverages our previously developed mammalian cell OG:A repair assay, recent advances in creating large saturation mutagenesis libraries of gene expression constructs, CRISPR and recombinase genome engineering approaches, and long-read DNA sequencing technology. Specifically, this project will entail developing methods for generating a large library of MUTYH variants that can be expressed in human cells. Cells will then be transfected with an OG:A fluorescent reporter to determine the extent of MUTYH-mediated repair and then sorted based on repair capacity. Analysis of the MUTYH variant constructs in specific sorted categories coupled with statistical data analysis will provide a quantitative assessment of the OG:A repair capacity (and thus the cancer-causing potential) of the MUTYH variants. The proposed project will entail analysis of a subset of possible MUTYH variants with the idea that once validated, this approach will provide a basis for analysis of all possible variants. Moreover, the approach may be readily adapted for the analysis of other BER cancer-associated variants.

Structure-guided ADAR1 inhibition to expand immunotherapy

Host Campus: Davis
Lead Investigator: Andrew Fisher
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,991

Abstract:
Treating cancer by employing immunotherapy checkpoint inhibitors has shown remarkable promise recently. This therapy has proven effective in treating diverse solid tumor types, including melanoma, non-small cell lung cancer, and many others. Immune checkpoint inhibitors are designed to block inhibitory immune signals and restore immune responses against tumor cells resulting in tumor eradication. However, most patients do not respond to immunotherapy, or develop therapeutic resistance because of mutations in the interferon pathway. A number of recent publications have identified that an RNA editing enzyme, ADAR1, plays an important role in both immune checkpoint and interferon pathways, and depleting or inactivating the enzyme sensitizes tumors to immunotherapy and overcomes resistance to the checkpoint blockade. ADAR1, or adenosine deaminase 1 acting on dsRNA, edits the RNA by changing adenosine to inosine (A-to-I), thereby altering its hydrogen bonding capacity, which will influence interactions with other RNA molecules including tRNA, resulting in potential codon changes. A-to-I editing has also been proposed to prevent the cytoplasmic dsRNA sensor PKR (protein kinase R) from erroneously recognizing endogenous dsRNA as foreign and triggering the interferon pathway. Knowing the atomic structure of human ADAR1 can lead to a better understanding of its function and aid in the development of inhibitors that, if administered with immunotherapy treatment, can increase the efficacy in immune checkpoint blockade therapy. Our laboratory recently determined the atomic resolution crystal structure of human ADAR2 complexed with RNA, which has furnished significant insight into its function. Human ADAR1 is 40% identical in protein sequence to ADAR2. This proposal aims to employ similar strategies used on ADAR2 structure determination, including incorporating nucleotide analogs to capture the protein-RNA complex formation, to uncover the structure of human ADAR1. This structure will drive inhibitor discovery trials by recruiting both virtual and activity-based screening algorithms to identify lead compounds. If successful in identifying an ADAR1 inhibitor, it will expand the number of cancer patients that can successfully be treated with checkpoint immunotherapy.

Detecting tumor-specific exosomes on a chip

Host Campus: Davis
Lead Investigator: J. Sebastian Gomez Diaz
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,999

Abstract:
Sensitive detection of circulating tumor-associated exosomes (TEXs) and related extracellular vesicles (EVs) may improve strategies for ovarian cancer (OvCa) detection and monitoring because (i) they represent stable and protected biomarkers in blood circulation and (ii) their composition, including surface protein markers, is tissue and pathology-specific. To achieve the best sensitivity for detecting low-frequency TEXs in early stage cancer patients, innovative high-resolution tools are needed. The cornerstone of this multidisciplinary project is to develop an extremely sensitive and affordable on-chip bio-sensing platform able to accurately detect the presence of EVs including TEXs in microseconds, with crucial implications for early detection of OvCa. We have previously demonstrated that our sensor, which relies on a novel transduction mechanism that merges tailored optical and nanomechanical resonances, outperforms at room temperature the sensitivity of the best commercial FTIRs over a narrowband, and here propose to apply this novel technology to bio-sensing for the first time.

To accomplish this objective, we will first isolate circulating EVs using density-gradient ultracentrifugation from human OvCa patient plasma. We are currently collecting more than one hundred plasma samples per week from women suspected of OvCa malignancy through the UCDCC Biorepository resource. Second, we will be capturing EV subsets on our innovative sensors according to multiplexed capture agents like known general exosome-specific and also cancer-exosome-specific molecules, including the recently reported potent OvCa-binding peptide, LXY30. This enrichment method will provide unprecedented sensitivity towards TEX subpopulations. Third, we will use the nanomechanical resonance properties of hundreds of individual detectors, tuned to particular intrinsic EV-specific vibrating frequencies, to label-free sensitively detect small numbers of EVs. Fourth, we will apply custom nanomaterials composed of gold nanoparticles (NPs) and nanorods (NRs) coated with EV-targeting agents to further identify subpopulations of detector-captured EVs. We expect the detected signatures to be significantly more sensitive and specific than the current gold standard diagnostic approach, which may accelerate clinical cancer diagnostic platforms for a wide range of cancers.

Prognosis of prostate cancer using multi-omics datasets

Host Campus: Riverside
Lead Investigator: Zhenyu Jia
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,500

Abstract:
Effective approaches are urgently needed to distinguish aggressive prostate tumors from the indolent forms. The currently used commercial tests, including Prolaris®, Decipher® and OncotypeDX GPS®, provide choices of treatment based on patients' individual disease states. However, the predictive abilities for these tests need to be increased to meet clinical need for resolving overtreatment which continues to be a health issue in prostate cancer management owing to the negative and unnecessary side effects. In these commercial tests, only a few dozens of genes are used for the perdition of disease outcomes. We have demonstrated that inclusion of many minor predictors, e.g., thousands of DNA markers, in genomic prediction models can substantially improve the trait predictability in crops. Therefore, we hypothesize (1) A prognostic model using whole-transcriptome metrics as predictors will outperform the commonly employed models which only consider major gene expression. (2) The prognostic power will be further increased if other omics predictors are also factored into the model. We will test these two hypotheses by leveraging The Cancer Genome Atlas (TCGA) data and two validation datasets which are publicly available. The best linear unbiased prediction (BLUP) methodology will be applied to the TCGA data to develop an optimal prediction model based on the selected multi-omic variables. The performance of the new multi-omics prognostic model will be verified by rigorous cross validation and by the validation datasets. Possible combination of clinical predictors with the selected multi-omics predictors will be investigated to further increase the predictability of the prognostic model. All the proposed analysis may be conducted on high-performance personal computers in the PI's lab. The high-performance computing facility at UC Riverside, including the main compute cluster with ~3600 CPU cores, 30TB of total RAM and the latest Infiniband interconnect, will be available for this project. The successful completion of the project will (1) prove the concept that prediction models using multi-omics datasets provide improved prognostic power in prostate cancer, and (2) produce a prognostic model that may be translated to clinical management of prostate cancer.

Meta Analytic Methods in the Presence of Competing Risks

Host Campus: San Francisco
Lead Investigator: Ann Lazar
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Meta-analysis of cancer trials frequently use recurrence and overall mortality (death due to any cause) as the primary endpoints. The typical statistical methods for these calculations are often based on logrank tests. For example, the Early Breast Cancer Trialists' Collaborative Group (EBCTC) analyzed 10 trials of 4756 women with early breast cancer using these logrank methods to assess recurrence and mortality endpoints (EBCTC, 2018). However, censoring competing risks (e.g., death) is a key factor in making these standard logrank meta-methods "work right", but unfortunately may be providing incorrect answers to important questions.

An unbiased approach recently developed to tackle this issue for analysis of individual studies is the Fine and Gray's competing risks approach. To our knowledge, however, this approach is not available for meta-analysis. Unfortunately, we lack meta-analytic strategies that can be used to accurately assess endpoints in the presence of competing risks.

We propose to:

Aim 1: Develop a meta-analytic competing risks approach that hinges on the theoretical developments of the Fine and Gray methodology. Using simulations or computer-generated artificial data in which we can impose known associations, we will assess the performance of this approach, including bias, type I error rate and statistical power.

Aim 2: Demonstrate the effectiveness of the meta-analytic method in eight Phase III randomized controlled clinical trials. We will illustrate the approach from Aim 1 using data from the Breast Cancer Data Mart (BCDM): 5,727 individual patient data, 1368 breast cancer recurrence events in the presence of competing events, 1581 deaths.

Aim 3: Disseminate results by developing available software and documentation. Statistical software and associated documentation, including tutorials, will be developed and disseminated to provide cancer researchers with the tools needed to implement meta-analysis in the presence of competing risks.

Biosynthesis of Phytosteroids as Novel Chemotherapies

Host Campus: Riverside
Lead Investigator: Yanran Li
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Cancer, as one of the most deadly diseases, is causing over 1 million deaths in the US, and over 8 million deaths worldwide annually. Although many novel treatment strategies have been developed these days, chemotherapy keeps playing a major role in the treatment of many cancers. There are two major disadvantages when employing chemotherapy treatment: 1) existing anticancer drugs are toxic to many normal cells in addition to cancer cells, and thus cause a long list of adverse effects; and 2) under many circumstances, cancer cells can develop resistance towards the anticancer drugs through mutations. Thus, anticancer agents of higher specificity towards cancer cells with fewer side effects towards normal cells are desired.

Plant-derived secondary metabolites have been an important source of anticancer agents, such as taxol and vinblastine. In addition, plants' synthetic potential remains largely under-exploited to date, and thus serves as one significant resources for the discovery and development of new types of anticancer drugs for improved and less toxic cancer treatment. Phytosteroids are a large group of phytochemicals. Among this group, many were shown to efficiently inhibit growth of many human cancer cell lines with no and minimal effect on the growth of normal cells, such as withaferin-A (withanolides) and 24-epibrassinolide (brassinosteroids). Phytosteroids thus serve as a group of promising leads toward the development of new anticancer drugs with few side effects.

We propose to elucidate, transform, and engineer the biosynthetic machinery of phytosteroids in baker's yeast through functional genomics and synthetic biology. Further metabolic engineering and combinatorial biosynthesis will enable the efficient production of phytosteroids of verified anticancer activities, and many more that were not isolatable or natively synthesized from nature. The project will be divided into three phases: 1) Elucidation of the biosynthesis of brassinosteroids and withanolides in yeast; 2) Optimization of the P450-rich biosynthetic pathways through metabolic engineering, protein engineering, and fermentation engineering; 3) Pathway engineering and protein engineering for the synthesis of novel natural and unnatural phytosteroids. The phytosteroids synthesized from this project will be eventually examined and evaluated in collaborating laboratory.

The role of PARP-induced stress granules in tumor survival

Host Campus: Los Angeles
Lead Investigator: Melody Man Hing Li
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
The tumor microenvironment changes dynamically and requires tumor cells to adapt to various stressors, such as hypoxia and nutrient starvation. These stressors trigger the assembly of cytosolic stress granules (SGs), sites of translation repression, leading to tumor growth and metastasis. Increasing evidence supports that induction of SGs upon exposure to oxidative stress can lead to tumor cell resistance to chemotherapy-mediated cytotoxicity, although the mechanism is not clear. SGs protect tumor cells likely by translation inhibition and inactivation of pro-apoptotic factors. Characterization of host proteins that catalyze SG assembly is crucial for understanding the anti-apoptotic role of SGs in cancer.

Poly(ADP-ribose) polymerases (PARPs) are important for nuclear processes. PARP1 and the tankyrase PARP5a have been extensively studied for their cancer-relevant functions in DNA repair, telomere homeostasis and Wnt signaling. However, the rest of the PARP family is poorly characterized. PARP5a, -12, -13, -14, and -15 are recently reported to localize to cytosolic SGs and to modulate the kinetics of SG assembly under arsenite-mediated oxidative stress. Given that some of the PARP inhibitors used in clinic demonstrate broad activity against multiple PARP proteins, we need a better understanding of the full spectrum of PARP-dependent cellular processes.

The goal of this proposal is to elucidate how PARP-induced SGs promote tumor cell survival, which will shed light on SG targeting as a therapeutic strategy. Given that the catalytic activity of PARPs is required for SG assembly and ADP-ribosylation can significantly change the function of the acceptor protein, we hypothesize that ADP-ribosylation of pro-apoptotic factors by SG-specific PARPs promotes tumor survival. In the first aim, we will determine the effects of ADP-ribosylation on translation and tumor cell survival. In the second aim, we will identify PARP substrates under oxidative stress. The proposed work will lead to identification of potential ADP-ribosylation targets that regulate cell proliferation, which will help prioritizing candidate genes for follow-up studies and provide insights into the mechanism of tumor resistance to apoptosis. Findings from this proposal will lay the groundwork for a more comprehensive and mechanistic study of the stress-coping mechanisms of tumor cells.

Role of lncRNA DDRLNC1 in DNA repair

Host Campus: Riverside
Lead Investigator: Xuan Liu
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Our recent genome-wide profiling of Poly(A) RNA in response to DNA damage identified a long non-coding RNA (lncRNA), termed DNA damage response long non-coding RNA 1 (DDRLNC1). Upon DNA damage, DDRLNC1 exhibits 5-fold increase in RNA level and alternative splicing. The Cancer Genome Atlas (TCGA) analysis revealed that DDRLNC1 level was significantly altered in a large number of tumors across many different cancer types. To understand its molecular mechanism of action, we performed RNA pull-down followed by MS analysis and identified XRCC5 (Ku70) and XRCC6 (Ku80) as the top DDRLNC1-associated proteins, supporting its role in double-strand break (DSB) repair by non-homologous end joining (NHEJ). To establish its functional significance, we deleted DDRLNC1 by CRISPR-Cas9 and observe increased level of unrepaired DNA upon DNA damage. Because NHEJ often promotes inaccurate re-ligation of DSB, it is believed to play a role in increasing genomic instability. Importantly, inhibition of NHEJ has been shown to play a critical role in radio-sensitization. Our working hypothesis thus is that, through interacting with Ku70 and Ku80, DDRLNC1 enhances NHEJ, which promotes error-prone DNA repair and reduces therapeutic responsiveness of cancer cells. To test our hypothesis the following two specific aims will be proposed. In Aim 1, we will study how DDRLNC1 regulates NHEJ repair. In Aim 2, we will address whether this regulation contributes to therapeutic responsiveness of cancer cells.

Impact of sex hormones on melanoma development

Host Campus: Irvine
Lead Investigator: Feng Liu-Smith
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Developing effective prevention strategies based on full knowledge of risk factors is urgently needed as melanoma incidence continues to increase despite the public effort on UV education in the past decades. In the past the sex difference in melanoma has always been attributed to differential sex-associated UV behaviors. Our epidemiological studies have recently revealed that the female sex itself is an independent risk factor for melanoma at younger age as compared to male sex. A bimodal mechanism of melanomagenesis is proposed to explain the sex- and age-differentiated melanoma risk: melanoma diagnosed at older age is mainly attributed to solar UV exposure with sex as a modification factor; while sex independently and directly contributes to early onset melanoma in addition to UV exposure. Our laboratory study suggested that testosterone enhanced DNA damage induced by solar-simulated radiation in normal human melanocytes, and estrogen induced differential ROS accumulation in melanocytes of different genders. Our pilot case-control study suggested that salivary testosterone and estrogen levels exhibited an age- and sex-differentiated association with melanoma. Hypothesis: estrogen and testosterone play crucial roles in early onset melanoma in women and men, respectively: estrogen promotes melanomagenesis via ROS in young women and testosterone protects young men via enhancing UV-induced DNA damage repair. Sex hormones play less roles and UV play more important role in late onset melanoma. In collaboration with clinicians and an experienced epidemiologist, we propose two specific aims: Aim 1: validate the age- and gender-specific associations of estrogen and testosterone levels with melanoma risk using a case-control study design. Aim 2: investigate the molecular mechanism of estrogen and testosterone in DNA damage repair in vitro in NHMs, and compare gender differences in a set of NHMs. This study is significant and novel as it is based on a new melanoma model, new discovery of salivary sex hormone association with melanoma and new role of testosterone in DNA damage in melanocytes. Completion of this study will unveil the role of sex hormone in melanoma development, and may lead to paradigm change of future prevention strategies based on hormone levels which are modifiable by life style factors such as diet and physical activities.

Activating anti-tumor immunity through the phosphatase SHP1

Host Campus: San Francisco
Lead Investigator: Clifford Lowell
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
The protein tyrosine phosphatase SHP1 (gene name Ptpn6) restricts the activation of a number of immune cell types, including neutrophils, macrophages, dendritic cells, B- and T-lymphocytes. SHP1 reverses immune cell activation by dephosphorylating intracellular signaling intermediates in a number of cell activation pathways. Mice with a spontaneous mutation in Ptpn6, which results in loss of SHP1 protein, develop skin and lung inflammation as well as systemic autoimmunity. In humans, mutations in PTPN6 are associated with chronic inflammatory skin diseases. Our laboratory has studied the immune cell types and intracellular signaling pathways regulated by SHP1 in the setting of inflammation and autoimmunity using inducible and conditional gene knockout mice.

Since SHP1 limits normal immune responses it may also function to restrict the immune response to cancer. Using our SHP1-deficient mouse models, we are in the position to test the hypothesis that SHP1 also restricts anti-tumor immunity. Study of anti-tumor immunity is a new area for our group; this proposed work will be carried out in collaboration with cancer researchers at UCSF.

We propose to challenge mice containing an inducible deletion of SHP1 (Ptpn6flox/floxROSA26-CreERT2) or conditional knockout animals that lack SHP1 in only neutrophils, monocytes/macrophages, dendritic cells or T-cells, with various well-studied syngeneic murine tumor lines (B16 melanoma, MC-38 colon cancer, EO77.1 mammary cancer). We will then monitor tumor growth and tumor immune responses in the wild type versus SHP1-deficient animals. In a single pilot experiment, inducible loss of SHP1 (in Ptpn6flox/floxROSA26-CreERT2 animals treated 6 days before tumor challenge with tamoxifen to cause Ptpn6 deletion) resulted in a significant delay in growth of MC-38 cells. We will also test whether deletion of SHP1 enhances the efficacy of immunotherapies (such as anti-PD1 checkpoint inhibitors or various anti-tumor monoclonal antibodies) directed against these tumors. If successful, this will lead to future studies to determine the mechanisms (the cell types and signaling pathways) by which SHP1 restricts tumor immunity. This work could also identify SHP1 as a new immunotherapeutic target in cancer.

Ectodysplasin Receptor in breast density and breast cancer

Host Campus: Berkeley
Lead Investigator: Kunxin Luo
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,497

Abstract:
It is well established that women of Asian descent have denser breast tissue (a risk factor for breast cancer) compared to other women world-wide but they have lower rates of breast cancer. However, the molecular mechanism underlying the relationship between breast density and breast cancer risk has never been determined. I am proposing a pilot study to determine the causal mechanism of the breast density and breast cancer risks in different human populations.

Evidence from across palentological, biological and agricultural sciences suggests that the ectodysplasin pathway and the Ectodysplasin Receptor (EDAR) determines ectodermal structures such as hair, teeth, sweat gland and mammary gland. In particular, the 370A variant of EDAR gene, which is enriched in Native Americans and people of Asian descent, underwent strong positive selection about ~30,000 years ago and is now believed to induce increases in mammary branching density in virgin mice, implicating its role in regulating breast density. However, its potential function in mammary gland development has never been studied in detail. We hypothesize that EDAR 370A is the critical causal link between denser breast tissue and the lower breast cancer rate observed in Native American and Asian women. To test this, we will generate a knockin mouse expressing human EDAR V370A in the mammary gland and cross it with a breast cancer mouse model to determine whether this allele affects breast cancer occurrence and progression. The impact of EDAR V370A on the activities of various signaling pathways known to be involved in breast cancer will be examined. Our study may open up an entirely new area of breast cancer research and identify new anti-cancer drug targets.

My past research focus is signal transduction and breast cancer using both mouse models and human cancer cell lines. This proposed project allows me to enter a new research direction to understand the factors influencing the mammary gland structures during early development and how this may contribute to breast cancer risk later. This project will be a collaborative effort with Dr. Leslea Hlusko, a world-class expert in human evolution and biological variation focusing on ectodermally-derived structures.

Linking the Circadian Clock and Cancer

Host Campus: Irvine
Lead Investigator: Selma Masri
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
The circadian clock controls several physiological, endocrine and metabolic processes that operate to maintain organismal homeostasis. These biological rhythms are self-perpetuating oscillations that are maintained within a 24-hour periodicity and are synchronized by external environmental cues such as light, temperature and food intake. Several lines of evidence undoubtedly suggest that disruptions in circadian rhythms results in numerous physiological disorders, including cancer. At the organismal level, genetic mutations in the circadian clock machinery accelerate tumorigenesis and this has been demonstrated in mouse models of leukemia/lymphoma, hepatocellular carcinoma, lung adenocarcinoma and osteosarcoma. At the molecular level, a crosstalk between the circadian clock and several oncogenic signaling pathways has been reported. Up-regulation of MYC has been shown to disrupt circadian gene expression and therefore perturb circadian glucose and glutamine metabolism in cancer cells. Conversely, the circadian clock has also been shown to target and degrade MYC, thereby inhibiting MYC-dependent proliferation. Additionally, the beta-catenin pathway has been reported to disrupt circadian gene expression, yet how clock disruption mechanistically drives enhanced beta-catenin signaling remains undetermined. We aim to further elucidate the molecular mechanisms related to the bi-directional crosstalk between the circadian machinery and cellular signaling pathways involved in survival and proliferation. Our work has the potential to open new avenues for therapeutic intervention targeting the circadian clock for the treatment of several cancers.

YAP in "simple & robust" tumor/stroma models of in PDAC

Host Campus: Riverside
Lead Investigator: Joshua Morgan
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Pancreatic ductal adenocarcinoma (PDAC) has one of the highest mortality of common cancers, with a five year survival rate of ~6%. Indeed, despite being the 11th most common cancer, it is the 5th most deadly. Further, the burden disproportionately falls on minority populations, with African American patients having 50-90% higher incidence and mortality, even after accounting for access to care. These sobering statistics underscore the need to identify novel therapeutic targets. Unfortunately, therapeutic progress in PDAC is hindered by the complex interactions between the tumor and its microenvironment, especially involvement of stromal cells in both restraining and encouraging tumor growth. Efforts to target tumor cells or their molecular signaling pathways can have unexpected effects in the stromal population. As a prime example, inhibiting Hedgehog (Hh) signaling proved effective in several PDAC models; however, this resulted in negative or negligibly positive gains clinically. Further research demonstrated that in addition to the roles Hh plays in tumor cell proliferation, Hh is a key component of restraining tumor growth by paracrine signaling with surrounding stromal cells. Similarly, chemotherapeutics that inhibit tumor growth in models can lead to senescence of stromal cells. A key phenotype of these senescent cells is to release a cocktail of cytokines into the microenvironment, encouraging paracrine angiogenesis and tumor metastasis. Preliminary data demonstrate that both Hh and the paracrine effects of senescence are regulated through the protein YAP, which was recently correlated with PDAC mortality but whose mechanistic roles are poorly understood.

Despite the importance of tumor/stroma interactions, organotypic models of PDAC remain limited to specialist labs. We propose to develop and validate "simple & robust" organotypic PDAC models that are cost-effective and easy to use, enabling adoption by non-specialist researchers. As key validation of these models, we will demonstrate the clinically relevant tumor/stroma interactions described above and further test the novel hypothesis that YAP regulates these interactions in two independent Aims. In Aim 1, we determine if YAP activity is required for the paracrine impacts of stromal senescence. In Aim 2, we determine if stromal YAP activity is activated by Hh inhibition to encourage tumor growth.

Regulation of cell proliferation by Unkempt

Host Campus: Riverside
Lead Investigator: Jernej Murn
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Neuronal differentiation presents arguably one of the most extreme cases of cell specialization in which fast expanding neural progenitors abruptly exit cell cycle to generate post-mitotic neurons. What instructs neuronal precursors to stop dividing and adopt a bipolar morphology is largely unknown. Understanding of the underlying mechanism would not only shed light on the balance between proliferation and differentiation during neurogenesis, but would have broad implications for tumorigenesis, where such balance is commonly disrupted. We previously identified a conserved RNA-binding protein (RBP), Unkempt, that is induced during early neurogenesis and we characterized its unique role in induction of the bipolar neuronal morphology. Unkempt-driven morphogenesis is accompanied by a sudden block of cell proliferation, an activity that is conserved across animal species and can be recapitulated in cells on non-neuronal lineages. Our preliminary studies demonstrate that Unkempt potently activates gene transcription, which is required for its antiproliferative as well as morphogenetic roles. We identify a non-coding RNA, 7SK, and multiple chromatin-associated proteins as candidate mediators of Unkempt's transcriptional activity. We further find that Unkempt is a direct target of its negative regulator, the mTOR kinase, which specifically phosphorylates the transcriptionally active domain of Unkempt. How Unkempt-driven gene transcription is coupled to its tumor-suppressive activity is unclear. Here, we propose to decipher the mechanistic basis of Unkempt-induced gene transcription to understand how it may be linked to cell cycle exit during neurogenesis and how it may antagonize expansion of non-neuronal cells. Specifically, we will (1) investigate the chromatin association of Unkempt and its impact on gene transcription, and (2) interrogate the regulation of Unkempt's transcriptional activity by the mTOR kinase. These studies will shed light on the regulation of the balance between cell proliferation and differentiation during development with important implications for cancer, where this balance is typically compromised.

Investigating the Roles of the ER Regulation in Mitosis

Host Campus: San Diego
Lead Investigator: Maho Niwa
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
The importance of accurate and coordinated cell cycle division is underscored by human diseases such as cancer that are caused by uncontrolled cell division. Thus far, studies of cell cycle checkpoints that ensure proper cell division have centered around chromosome replication and segregation. Little is known about the existence of cell cycle checkpoints that monitor the fidelity of cytoplasmic organelle divisions. In recent work, we discovered a cell cycle checkpoint that ensures inheritance of functional endoplasmic reticulum (ER) during cell cycle in S. cerevisiae. The ER, one of the biggest organelles in the cytoplasm, plays vital roles in the production of secretory proteins, lipid biosynthesis, and detoxification. Importantly, the ER cannot be generated de novo. We found that ER stress blocks ER inheritance into the daughter cell, leading to a halt in the cell cycle by the mislocalization of the septin ring away from the bud neck and cytokinesis block. A failure to halt the inheritance of stressed ER causes cell death, highlighting the importance of this checkpoint. The major goal of this proposal is to investigate in detail how cells ensure functional quality of the ER during the cell division.

As the nuclear membrane breaks down during the cell cycle, nuclear membrane proteins and lipids are dispersed into the ER and are retrieved back to the nuclear membrane as it reassembles during anaphase. We obtained preliminary results revealing that the reassembly of the nuclear membrane is delayed in mammalian cells in response to ER stress, leading to the cytokinesis delay by mislocalizing septin rings from the midbody. In some cancer cells, ER stress and the components of the unfolded protein response, a signaling pathway for establishment of ER homeostasis, are highly induced. Thus, we hypothesize that the functional homeostasis of the ER impacts nuclear membrane assembly/disassembly, which may provide a basis for the cell division checkpoint that ensures divided cells have functional ER. In this proposal, we will (1) dissect the molecular events and mechanisms leading to the ER stress-induced delay in nuclear membrane reassembly, (2) interrogate the impact of ER stress on other mammalian cell cycle stages, and (3) evaluate the impact of modulating unfolded protein response component activities on ER stress-affected cell cycle events.

Identifying vulnerabilities in drug-resistant cancer

Host Campus: Berkeley
Lead Investigator: James Olzmann
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,849

Abstract:
The emergence of resistance mechanisms in response to targeted chemotherapies remains an incredibly important and unaddressed challenge in cancer treatment. During treatment, a small fraction of cells often evades first-line chemotherapies, resulting in cells in a drug-resistant "persister" state that have the potential to initiate relapse and growth of a drug-resistant tumor. Exciting recent studies discovered that persister cells are sensitive to a regulated form of cell death called ferroptosis. These results raise the possibility that activation of ferroptosis is a viable approach to kill persister cells. Unfortunately, our understanding of the mechanisms of ferroptosis in persister cells remains limited. Moreover, whether there are additional vulnerabilities in persister cells that can be therapeutically exploited remains an open question.

We have developed systems-level strategies to uncover mechanisms of cancer cell drug resistance. For example, exploiting a whole genome CRISPR synthetic lethal screen, we recently identified a new protective mechanism involving the oxidoreductase AIFM2 that suppresses lipid peroxidation and promotes ferroptosis resistance in lung cancer cells. These preliminary findings support the utility of our functional genomic platforms to identify new therapeutically relevant targets in lung cancer.

Our proposed research exploits our genetic strategies to uncover and drug novel vulnerabilities in cancer persister cells. Our research examines cell death pathways (CSO1.1) and the biology of persister cancer cells (CSO1.4). We will directly examine the hypothesis that AIFM2 protects drug-resistant cancer cells from ferroptosis and we will implement whole genome CRISPR screens to identify novel vulnerabilities in persister cells. Together, these complementary approaches will uncover new mechanisms of drug resistance and will identify therapeutically relevant targets that can be targeted to kill cancer persister cells.

Mechanisms of Tissue-Resident Immunity to Metastasis

Host Campus: Los Angeles
Lead Investigator: Timothy O'Sullivan
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,927

Abstract:
Metastasis is the leading cause of cancer-related deaths. Research from the past decade has demonstrated the exciting potential of harnessing a patient's immune system to prevent or delay tumor progression and metastasis. Although immunotherapies targeting adaptive lymphocytes (i.e. antigen-specific T cells) have proven successful in certain circumstances, many cancer patients ultimately do not respond and can suffer complications due to unrestrained immune responses. Thus, there is an increasing need to understand the fundamental mechanisms utilized by the host immune system to suppress tumor formation and metastasis in order to develop more effective therapeutic strategies. Innate lymphoid cells (ILCs) are tissue-resident lymphocytes that are rapid producers of both proinflammatory and regulatory cytokines in response to local injury, inflammation, pathogen infection, or commensal microbiota perturbation. Our work has shown that type 1 ILCs (ILC1) are present in all organs analyzed in mice, and are the first and most potent lymphocyte producers of interferon (IFN)-gamma in initial virally infected tissues in response to local dendritic cell (DC) production of interleukin (IL)-12. However, the role of tissue-resident ILCs during tumor metastasis to distal organs remains unknown. We have found that genetic deletion of both liver resident ILC1 and circulating natural killer (NK) cells leads to increased liver metastasis of melanoma tumor cells. Furthermore, we have found that the absence of liver-resident ILC1 responses leads to suboptimal recruitment and activation of NK cells during viral infection. In this proposal, we will build on this preliminary data by investigating the hypothesis that liver-resident ILC1 prime endogenous anti-metastatic innate immune responses through early production of IFN-gamma. We will investigate this hypothesis through two aims: Aim 1 – Determine whether liver ILC1-derived IFN-gamma is required for decreased metastasis and NK cell recruitment to the liver in vivo; Aim 2 – Investigate whether liver ILC1 are required for optimal DC activation during tumor metastasis in vivo. Our work will challenge traditional paradigms that only target circulating lymphocytes for tumor immunotherapy, and discover novel mechanisms by which tissue-resident immunity restrains metastasis.

Structural insight into METTL13-mediated eEF1A methylation

Host Campus: Riverside
Lead Investigator: Jikui Song
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Protein synthesis is one of the essential cellular processes that control cell survival and proliferation. To adapt to various cellular environments, the activity of translation machinery is fine-tuned by a multifaceted regulation, including a wide array of posttranslational modifications (PTMs). Dysregulation of translation in cancers results in boosted global protein synthesis or selective production of oncogenic proteins, which in turn promotes tumor growth and metastasis. Recent evidence has revealed that methylation of the lysine residues of eukaryotic elongation factor 1 alpha (eEF1A), which delivers aminoacyl-tRNAs to the ribosome during mRNA translation, regulates translation dynamics in cells. For instance, the N-terminus and lysine 55 (K55) of eEF1A are both subjected to specific methylation by methyltransferase (MTase)-like protein 13 (METTL13). Upregulation of METTL13 and K55-methylated eEF1A levels in lung adenocarcinoma (LAC) and Pancreatic ductal adenocarcinoma (PDAC) promotes translational output and negatively correlates with patient survival. METTL13 belongs to a novel protein methyltransferase family, harboring two tandem methyltransferase domains. The N-terminal MTase domain of METTL13 mediates the di-methylation of eEF1A K55, while the C-terminal MTase domain is responsible for the N-terminal tri-methylation. Due to the lack of the structural information, how these two domains achieve specific eEF1A methylation is currently unclear, which hampers the development of specific inhibitors against this emerging therapeutic target. In this application, we seek to determine the structural basis of METTL13-mediated methylation of eEF1A. In Aim 1, we propose to determine the atomic structure of METTL13 free and in complex with eEF1A, thereby providing molecular details on the METTL13-eEF1A recognition. In Aim 2, we will perform structure-guided mutational and enzymatic analysis to determine the enzymatic mechanisms and functional cooperation between the two MTase domains of METTL13. Together, these studies will provide mechanistic details of the METTL13-mediated eEF1A methylation, setting a paradigm for the structure-function relationship of METTL13-like protein methyltransferases. Furthermore, the proposed study will provide a framework for the development of novel therapeutic agents against cancers.

New Methods for the Synthesis of Deuteriated Small Molecules

Host Campus: Merced
Lead Investigator: Benjamin Stokes
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Transition metal hydrides are reactive intermediates that are useful for the interception and subsequent transformation of unsaturated bonds and other functional groups. We recently introduced a powerful new method for nanoparticulate (heterogeneous) palladium-hydride ([Pd]–H) generation using water as an H atom donor in the presence of diboron(4) reagents, and demonstrated that this method could be used to hydrogenate a variety of unsaturated C–C bonds. Lately, we have improved our understanding of this reaction by developing a homogeneous variant of the reaction. Now with an arsenal of homo- and heterogeneous catalytic conditions at our disposal, we are primed to develop new reductive reactions of functional groups abundant in organic compounds. Our aims are to use the water/diboron(4) system to 1) develop complementary methods for catalyst-controlled Pd-catalyzed trans- and cis-selective semihydrogenation and semideuteriation of alkynes; and 2) develop a mild reductive dehalogenation of haloarenes using H or D atoms from water. Most prescription and over-the-counter medications (including cancer chemotherapeutics) are composed of small organic molecules, where 'small' typically means 15–30 carbon, nitrogen, or oxygen atoms, and 'organic' means molecules wherein the majority of non-hydrogen atoms are carbon atoms. The development of deuterium-containing small molecule organic pharmaceuticals is in its infancy, with the first notable deuterium drug analog (Austedo®) having received FDA approval in 2017 for the treatment of Huntington's disease. Despite the recent FDA approval of Austedo®, many breakthrough medications remain inaccessible due to limitations in chemists' ability to connect carbon and deuterium atoms efficiently and selectivity. The sustainable methods that we will develop will enable the construction of molecules containing one or more deuterium atoms selectively placed on specific carbon atoms, which is imperative for the development of the next generation of cancer chemotherapeutics.

Downregulation of XIST in Ovarian Cancer and Its Mechanisms

Host Campus: Irvine
Lead Investigator: Sha Sun
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
This project aims to elucidate the functional role of cancer-associated long noncoding RNA (lncRNA), in particular, lncRNA XIST (X-inactive specific transcript) in ovarian cancer. Genome-wide association studies in cancer have revealed that more than 80% of cancer-associated genetic variations occur in noncoding sequences of the human genome. In parallel, an increasing number of cancer transcriptomes have also shown thousands of lncRNAs differentially expressed in a variety of human cancers when compared to normal cells. These indicate that understanding lncRNA-associated oncogenic pathways should be an important part of understanding the genetic mechanisms in cancer. The lncRNA XIST has recently been reported to affect cancer metastases and tumor progression. But whether the dysregulation of XIST can directly drive cancer symptoms, what are the XIST-mediated oncogenic pathways, and whether the lncRNA can be therapeutically targeted for cancer biomarkers are unclear. In addition, since XIST is known as the master regulator of X chromosome dosage compensation between XX female and XY male mammals, how XIST is regulated and whether differential expression of X-linked genes may affect female cancer in particular, are notable questions not fully addressed.

Research in my lab has focused on the positive and negative regulators of XIST and, for the first time, reported detailed molecular mechanisms for the function of another lncRNA, Jpx, in activating Xist in mice. To investigate possible mechanisms of XIST regulation in cancer, we have used the cBioPortal for cancer genomics and observed significant downregulation of XIST correlated with higher neoplasm histological grades of ovarian cancer. We hypothesize that XIST, with its activator JPX, can function as tumor suppressors influencing oncogenic pathways related to proliferation and metastasis. We propose to identify the tumor growth relevant pathways in which XIST and JPX are associated within ovarian cancer. We also aim to define the genetic mechanisms underlying the loss of XIST in tumor cells. The outcomes will contribute to the functional annotation of cancer-associated lncRNA and impact future development of RNA biomarkers that may inform cancer diagnosis and treatment.

Samoan Healthy Eating and Active Living (HEAL)

Host Campus: Irvine
Lead Investigator: Sora Tanjasiri
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,910

Abstract:
Pacific Islanders (PIs) are disproportionately affected by the causes and contributing factors regarding cancer health disparities, as identified nearly 15 years ago by the U.S. Department of Health and Human Services (2004). Cancer is the leading cause of death for PIs, with obesity implicated as a causal factor in the onset of many cancers, including breast, colon, endometrium, esophagus, and kidney cancers. Unfortunately, relatively little is known about how to effectively prevent/reduce obesity among PIs who number 1.3 million in the U.S., 305,202 in California, and 88,050 in the San Francisco bay area. Responding to the National Cancer Institute's call for identifying factors influencing implementation of existing evidence-based interventions into community settings, we propose a multi-level, exploratory, implementation science pilot that applies the Consolidated Framework for Implementation Research (CFIR) to understand the factors associated with the potential adaptation to increase healthy eating and physical activity among Samoans in the bay area. The specific aims are to: 1) explore the organizational pre-implementation factors associated with program adoption among the parishes of the Samoan Congregational Christian Church of American Samoa in the Northern California region. We will conduct key informant (KI) interviews with two leaders from each of 12 parishes (representing 3,000 Samoan members) to understand their perceptions of the barriers and facilitators to EBI adoption; and 2) identify the individual nutritional intake and physical activity levels among members from one parish. We will survey n=70 Samoan adults at this parish to estimate the point prevalence of current diet and physical activity behaviors to inform power analyses in the future R01. The research team is comprised of two academics and two community leaders, all of whom have worked together in the past and have expertise in Pacific Islander health and community-based participatory research. This exploratory study is the essential precursor to the design of a larger implementation study, and the results will inform the development of a larger implementation research proposal in response to the NIH PAR Dissemination and Implementation Research for Health or similar R01 opportunity.

Roles of epigenetic regulators in bladder cancer progression

Host Campus: Santa Cruz
Lead Investigator: Zhu Wang
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $74,960

Abstract:
Bladder cancer is one of the most costly cancers to treat; yet our understanding of cancer progression in the bladder is lagging behind other organs. One big obstacle in the field is the difficulty of building relevant bladder cancer mouse models, since the bladder urothelial cells are extremely resistant to transformation by just one or two oncogenes and no good bladder-specific Cre line is available. TCGA analyses of muscle invasive bladder cancer have revealed the top recurrent mutated genes, and inactivating mutations in three epigenetic regulators KMT2D, KDM6A, and ARID1A are very common. Based on this finding and previous in vitro studies in the field, we hypothesize that these three epigenetic regulators are essential for preventing bladder cancer progression. To test it, we have developed a novel method of delivering DNA plasmids into the mouse urothelium through electroporation, and built a baseline p53 Pten mouse bladder cancer model by delivering the CRISPR/Cas9 plasmids targeting those two genes. Our preliminary histology and sequencing data showed that this approach can reliably generate bladder tumors with advanced-stage carcinoma in situ, and that the resulting tumors can be clonally derived from individual mutant cells. We will therefore build gRNA plasmids targeting various combinations of the three chromatin-remodeling genes, and co-deliver them with the p53 Pten plasmid. We will perform histological analyses of the bladder tumors for different groups of mice at early and later time points, and determine whether further mutating any combinations of Kmt2d, Kdm6a, and Arid1a genes will promote bladder cancer progression. In sum, by developing and validating a novel CRISPR-based cancer modeling approach, my lab is well positioned to do complex genetic modeling of bladder cancer in vivo and study the molecular mechanisms. With the CRCC seed funding, this study has promise of revealing a driver's role for key epigenetic regulators, and should lead to future NIH grant applications.

Investigating the in vivo functions of Septin 9

Host Campus: Merced
Lead Investigator: Stephanie Woo
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
SEPT9, a member of the septin family of cytoskeletal proteins, has been implicated in colorectal cancer (CRC); in fact, hypermethylation of SEPT9 DNA is the basis for an FDA-approved test for CRC. Despite these advances, the molecular mechanisms by which SEPT9 contributes to tumorigenesis is still unknown, partially due to a severe lack of in vivo studies. Our lab has recently found that the zebrafish homolog of SEPT9, sept9a, is specifically expressed in the endoderm – the precursor to the gastrointestinal (GI) epithelium. Here, we propose using the developing GI epithelium in zebrafish as an in vivo model to investigate the functions of sept9a. First, we will generate the necessary mutant, transgenic, and knock-in zebrafish lines to manipulate and assess sept9a function. Then, we will determine whether and how sept9a regulates cell adhesion and cell migration, two cellular processes relevant to both gut development and cancer.

Identifying cellular factors that inhibit telomerase

Host Campus: Davis
Lead Investigator: Lifeng Xu
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Telomerase directs the synthesis of new telomeric repeats during S phase of the cell cycle. As the vast majority of human cancers rely on telomerase for their ability to continuously proliferate, inhibition of telomerase function is deemed a potential cancer therapy strategy. In mammals, the six-subunit shelterin complex binds along telomeric DNA. Within the shelterin complex, the TPP1 subunit directly interacts with telomerase and plays an important role in recruiting telomerase to telomeres. It is unclear how other shelterin proteins, as well as the local telomere chromatin structure change, contribute to telomerase recruitment. It is also not known whether a separate activation step is needed for telomerase to start extending telomeres. Via gene editing, my laboratory has recently constructed an HCT116 human colon cancer cell clone containing a homozygous TPP1 mutation that disrupts the interaction between TPP1 and telomerase. Even though the cells still possess the intact telomerase enzymatic complex, telomerase cannot get to telomeres to extend them. As a result, the cells undergo progressive telomere shortening before suddenly entering into a persistent replicative senescence when their telomeres reach a critical length. Equipped with this cell line, we plan to perform a genome-scale CRISPR/Cas9 knockout screen to identify novel factors whose inactivation upregulate telomerase function and prevent cancer cells from entering into the telomere-based replicative senescence. We anticipate that the identified factors may include: inhibitors of telomerase recruitment/activation; inhibitors of alternative lengthening of telomeres; factors that mediate DNA damage repair; factors that promote senescence. We plan to extend this work into an NIH R01 proposal aimed at gaining mechanistic understanding of telomerase activity control. Knowledge obtained from this and follow-up study will help us understand how cells send signal to telomerase to turn it on and off at the right time and place. Novel telomerase inhibition strategy of cancer therapeutic potential may also be developed by modeling after negative regulators of telomerase.

A proteomic approach to study the SUMO pathways in cancer

Host Campus: San Diego
Lead Investigator: Huilin Zhou
Start Date: 1/1/2020 *End Date*: 12/31/2020 *Amount*: $75,000

Abstract:
Protein sumoylation, the covalent attachment of the Small Ubiquitin-like MOdifier (SUMO) to target proteins in cells, plays a major role in preventing genome instability; a hallmark of cancer. Interestingly, data procured by The Cancer Genome Atlas (TCGA) has identified numerous mutations affecting the enzymes that catalyze reversible sumoylation in humans. However, little is known about their substrates, thus, the biological consequence of these mutations has been unknown. A major bottleneck has been a lack of sensitive, accurate and robust proteome-wide technology to analyze protein sumoylation, which is broadly applicable for the study of cancer. Such a proteomic technology, once developed, has the potential to identify the molecular defects caused by cancer-associated mutations; thereby facilitating the development of new therapeutic and diagnostic strategies.

Protein sumoylation is highly conserved from yeast to humans. Using the model organism Saccharomyces cerevisiae, we have previously developed a proteome-wide technology to study the yeast SUMO pathway and obtained substantial insights into its enzyme-substrate relationship. More recently, we have adapted our proteomic technology to study the human SUMO pathway and generated a collection of mutant cell lines that are devoid of specific SENP enzymes. In the proposed studies, we will pursue three specific aims. First, we will apply our newly developed proteomic technology to identify substrates of the SENP family proteases, using a collection of mutant cell lines devoid of one or more SENP enzymes. This study is expected to provide the first SENP-substrate relationship in humans. Second, we will apply the TurboID approach to identify the transiently associated proteins of the SENP enzymes, allowing us to determine the direct SENP substrates and ultimately dissect their substrate specificity. Third, we plan to develop a broadly applicable proteomic approach to identify the SUMO-associated proteome in any cell, and then use this approach to characterize the role of the SENP enzymes in a panel of cancer cell lines that contain specific SENP alterations. Altogether, these studies aim to characterize the function and molecular mechanism of the human SENP enzymes; ultimately determining how their cancer-associated mutations affect sumoylation in cancer and impact the genesis of cancer.