UFHealth
CANCER CENTER

UFHCC Cancer Research Showcase

November 3rd, 2017

9:00am-4:30pm

CGRC Auditorium

ABSTRACT BOOK

UFHealth CANCER CENTER

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Showcase Agenda

9:00am- 9:10am	Welcome by Jonathan Licht, MD & Rolf Renne, PhD
9:10am- 9:35am	**Peter Ly, PhD**, Ludwig Institute for Cancer Research *"Rebuilding after catastrophe: Mechanisms of genomic rearrangements from cell division errors."*
9:35am- 10:00am	**Elise Fouquerel, PhD** University of Pittsburgh *"Local induction of oxidative base damage at telomeres: Deciphering the role of base excision repair in the preservation of telomere integrity."*
10:00am- 10:25am	**Mingyi Xie, PhD**, University of Florida *"MicroRNA biogenesis at the RNA polymerase II promoter proximal sites."*
10:25am- 10:50am	**Peter Choi, PhD**, Dana-Farber Cancer Institute *"Investigating the role of alternative splicing in cancer pathogenesis."*
10:50am- 11:20am	Coffee break
11:20am- 11:45pm	**Advaitha Madireddy, PhD**, Albert Einstein College of Medicine *"The multifaceted role of the Fanconi anemia tumor suppressor pathway in DNA replication."*
11:45pm- 12:10pm	**Mark Murakami, MD**, Dana-Farber Cancer Institute *"Overcoming resistance in BCR-ABL-rearranged acute lymphoblastic leukemia."*
12:10pm- 12:35pm	**Olga Guryanova, MD, PhD**, University of Florida *"DNMT3A directs therapeutic response in acute myeloid leukemia."*
12:35pm- 1:00pm	**Melike Çağlayan, PhD**, NIH/NIEHS *"Effects of oxidative stress on mammalian base excision repair."*
1:00pm- 2:00pm	Lunch
2:00pm- 2:35pm	**Keynote: Duane Mitchell, MD, PhD**, University of Florida *"Next generation immunotherapy approaches for brain tumors."*
2:35pm- 3:00pm	**Marco Ruella, MD**, University of Pennsylvania *"Resistance to chimeric antigen receptor T cell therapy."*
3:00pm- 3:25pm	**Xiaoyang Zhang, PhD**, Dana-Farber Cancer Institute *"Noncoding and coding genomic alterations converge to activate oncogenic transcription factors and promote tumorigenesis."*
3:25pm- 3:50pm	**Justin Brown, PhD**, Dana-Farber Cancer Institute *"The role of physical activity and body composition in colorectal cancer prognosis."*
3:50pm-4:15pm	**Janice Krieger, PhD**, University of Florida *"Delivering precision messages through the patient portal: Examining the efficacy of virtual providers for promoting cancer prevention."*
4:15pm-4:30pm	Closing Remarks

University of Florida Faculty Speakers

UF Faculty Speakers

Keynote Speaker: Duane Mitchell, MD, Ph.D.

Phyllis Kottler Friedman Professor in the Department of Neurosurgery and the State of Florida Endowed Chair of Cancer Research at the UF Health College of Medicine.

Dr. Mitchell serves as Director of Cancer Therapeutics & Immuno-Oncology at the UF Health Cancer Center and Co-Director of the Preston A. Wells, Jr. Center for Brain Tumor Therapy. Dr. Mitchell leads a comprehensive translational research program focused on brain tumor immunotherapy, and is principal investigator on a number of first-in-human immunotherapy clinical trials in pediatric and adult patients with brain cancer.

Olga Guryanova, MD, Ph.D.

Assistant Professor, Dept. of Pharmacology & Therapeutics

Dr. Guryanova's research is focused on the blood system, with a specific interest in hematologic malignancies. The overarching goal of her laboratory is to delineate the mechanisms of the cross-talk between epigenetics and chromatin organization, and how these processes contribute to the development of acute myeloid leukemia (AML), resistance to therapies, and clonal evolution. Ultimately, she would like to harness this mechanistic understanding to develop improved therapeutic approaches for leukemia.

Janice L. Krieger, Ph.D.

Professor, Dept. of Advertising/Health Outcomes & Policy
Director, STEM Translational Communication Center

Dr. Krieger is trained as a communication scientist, with expertise in precision messaging. Her research focuses on developing communication strategies for promoting awareness, understanding, and informed decision-making about cancer prevention and treatment behaviors. She is PI for an NCI funded study (1R01CA207689-01) investigating the use of virtual humans to deliver precision messages via patient portals and co-investigator on a number of NIH-funded projects. She directs the UF STEM Translational Communication Center (STCC), the only center of its kind dedicated to the development and dissemination of theory- and evidenced-based communication interventions. She also co-directs the CTSI Recruitment Center (U54TR001347).

Mingyi Xie, Ph.D.

Assistant Professor, Dept. of Biochemistry & Molecular Biology

The Xie Lab's research interest is to decipher biogenesis pathways that generate RNAs essential for gene expression in metazoans and associated viruses. Our focus now is on microRNA biogenesis, which generates ~22 nucleotide RNAs that modulate gene expression at the post-transcriptional level. In the near future, our research will integrate approaches from biochemistry, cell biology, virology and bioinformatics to study viral and cellular non-canonical miRNA biogenesis pathways. These pathways surprisingly incorporate several fundamental cellular machineries involved in processing other classes of RNAs and therefore can be exploited to delineate RNA biogenesis more broadly.

Invited Speakers

Justin Brown, PhD

Institution: Dana-Farber Cancer Institute, Boston, MA
Area: Population Sciences
K Award Project Title: INFLAMMATION; PHYSICAL ACTIVITY; AND COLON CANCER RECURRENCE AND SURVIVAL

The role of physical activity and body composition in colorectal cancer prognosis.

This presentation will introduce a research program which aims to demonstrate that lifestyle behaviors—such as the participation in physical activity and maintenance of a healthy body composition—reduce the risk of recurrent disease and mortality in patients with colorectal cancer. Three National Cancer Institute sponsored studies will be described. The first study is a phase II randomized clinical trial that examines the dose-response effects of physical activity on prognostic biomarkers in 39 patients with stage I-III colon cancer. The second study is a population-based cohort that examines the effects of muscle wasting on all-cause and colorectal cancer-specific mortality in 1,924 patients with stage I-III colorectal cancer. The third study is ongoing and contains two parts. Part one will quantify the extent to which physical activity mediates the relationship between systemic inflammation and recurrent disease and mortality in a national cohort of 2,526 patients with stage III colon cancer. Part two will use the results from the mediation analysis to inform the design of a randomized clinical trial of physical activity that will utilize measures of systemic inflammation as study endpoints in 100 patients with stage II-III colorectal cancer. After this presentation, attendees will be able to: 1) recognize the relevant lifestyle behaviors that have been associated with cancer prognosis and; 2) describe the physiologic mechanisms through which lifestyle behaviors may influence cancer prognosis. Patients with colorectal cancer consistently cite fear of recurrent disease as their chief health concern. Consequently, physicians often field questions such as “Should I exercise? Should I lose weight? What will improve my chances?” Unfortunately, patients soon learn there are few definitive answers to such basic inquiries. The long-term goal of this research program is to demonstrate that lifestyle behaviors improve clinically-meaningful endpoints, which will advance the management of colorectal cancer and empower patients with practical solutions to improve their outcomes.

Melike Çağlayan, PhD

Institution: National Institute of Environmental Health Sciences, Durham, NC

Area: DNA Repair

K Award Project Title: OXIDANT AND ENVIRONMENTAL TOXICANT-INDUCED EFFECTS COMPROMISE LIGATION IN DNA REPAIR

Effects of oxidative stress on mammalian base excision repair.

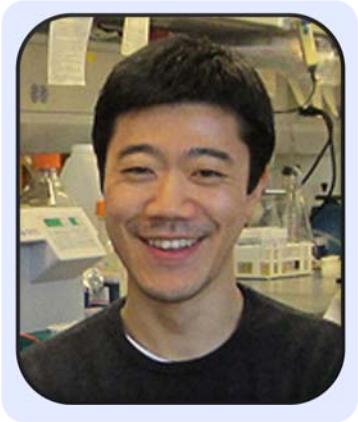
Oxidative stress from the combined effects of environmental and endogenous factors leads to accumulation of reactive oxygen species in cells and harmful consequences. Thus, oxidation of nucleotide bases in the DNA precursor pool and oxidation of DNA bases themselves have been proposed as triggers for the genomic instability and cell death observed after exposure to oxidative stress. Knowledge of how the mammalian cells responds to these genotoxic events is still lacking. One of the cellular responses to oxidative stress is repair of oxidative DNA damage by base excision repair (BER) that includes a coordinated sequence of enzymatic steps involving a channeling of BER intermediate from DNA polymerase (pol) β gap-filling to next DNA ligation step. However, pol β gap filling with an oxidized purine nucleotide leads to a block at the DNA ligation step. This pol β-mediated ligation failure depends on the base of the oxidized purine nucleotide inserted and identity of the template base during gap filling. Pol β active site mutations alter the ligation failure, pointing to a functional interaction between these enzymes, DNA ligase and pol β. Toward extending these results in a mouse fibroblast cell model, we observe more oxidative stress-induced cell killing as well as formation of DNA double-stranded breaks in the presence of pol β than in pol β null cells. The cytotoxicity is increased following gene deletion for the main oxidized nucleotide pool-sanitizing enzyme, MTH1; this effect likely involves oxidized purine nucleotide accumulation in the nucleotide pool and is consistent with pol β-mediated oxidized purine nucleotide gap filling and blocked DNA ligation during repair. Overall, our results suggest that a DNA strand break with an oxidized purine base at the 3'-margin represents an oxidative stress-induced stalled repair intermediate capable of triggering cell death.

Peter Choi, PhD

Institution: Dana-Farber Cancer Institute, Boston, MA

Area: Oncogenesis

K Award Project Title: INVESTIGATING THE ROLE OF RBM10-REGULATED ALTERNATIVE SPLICING IN LUNG TUMORIGENESIS



Investigating the role of alternative splicing in cancer pathogenesis.

The alternative splicing of RNA is an essential mode of gene regulation that greatly diversifies the transcriptomes of normal cells. However, this complex process is often dysregulated in human cancer and much remains to be understood about how aberrant splicing contributes to cancer-related phenotypes. We have explored the relationship between alternative splicing and cancer in two clinically-relevant contexts: the epithelial-to-mesenchymal transition (EMT) in breast cancer and treatment resistance in prostate cancer. EMT is a highly dynamic process that is thought to play an important role in tumor initiation and metastasis. Through a genome-scale ORF screen, we discovered that several splicing regulatory proteins, including QKI and RBFOX1, are sufficient to induce an EMT phenotype. By integrating transcriptomic and RNA-protein interaction data, we identified an alternatively spliced isoform of the actin-binding protein FLNB as a major effector and marker of the mesenchymal cell state in breast cancer. In prostate cancer, tumors often become resistant to castration therapy through expression of alternative isoforms of the androgen receptor (AR). We performed a genome-wide CRISPR screen using a novel reporter of AR splicing and identified several factors involved in RNA processing. We are currently investigating how these factors regulate AR expression and testing their potential as therapeutic targets for treating castration-resistant prostate cancer. Collectively, our work highlights how alterations in RNA splicing can play a significant functional role in driving diverse cancer phenotypes.

Elise Fouquerel, PhD

Institution: University of Pittsburgh, Pittsburgh, PA

Area: DNA Repair

K Award Project Title: DECIPHERING THE MECHANISMS OF PARP1 ACTIVITY IN TELOMERE INTEGRITY

Local induction of oxidative base damage at telomeres: deciphering the role of base excision repair in the preservation of telomere integrity.

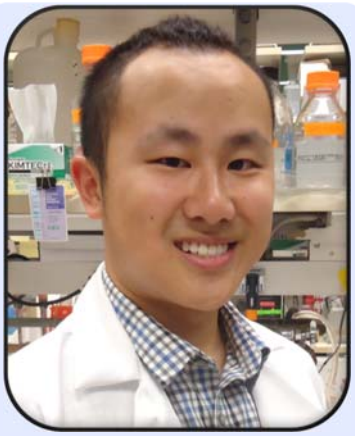
Telomere maintenance is essential for genome stability and for continued cell proliferation. Due to their specific TTAGGG repeat arrays, telomeres are highly susceptible to oxidative stress, which is shown to accelerate telomere attrition, and is associated with aging-related diseases and cancers. Base excision repair (BER) is responsible for the removal of oxidized bases. The effect of DNA damage on telomeres and particularly the role BER in telomere repair have been investigated by exposing human cells to conventional DNA damaging agents such as hydrogen peroxide, methyl-n-nitrosoguanidine, or ionizing radiation. However, these agents broadly affect the genome and can alter other cellular functions, thereby indirectly impacting telomeres and the global cellular response. Therefore, examining BER specifically at telomeres requires a novel technology that can induce DNA base damage locally. We developed a system that selectively generates singlet oxygen at telomeres, which reacts with DNA to produce 8-oxoguanine. This approach uses fluorogen activated peptides (FAP) which bind the fluorogen malachite green (MG). The FAP localizes to telomeres through a fusion with telomere binding protein TRF1. Singlet oxygen production is only activated when an MG derivative binds to the FAP, and the cells are exposed to 660 nm light. The significant advantage of this system lies in the inducible mechanism to generate DNA damage at specific sites within the genome. We generated two stable clones derived from telomerase positive cells (HeLa LT harboring long telomeres), and from osteosarcoma U2OS cells (which lengthen their telomeres through the ALT pathway). Using this system we observed the selective recruitment of BER proteins OGG1 and XRCC1 to telomeres after FAP-MG activation. XRCC1 recruitment was inhibited by pretreatment with PARP1 inhibitors BMN673 and ABT888. We also show that FAP-MG activation does not trigger direct double strand breaks at telomeres further demonstrating the specificity of this system. We find that repeated cycles of singlet oxygen generation at telomeres and recovery over a course of 18 days leads to telomere loss, micronuclei formation and mitotic aberrations. These results provide evidence for the local formation of 8-oxo-guanine at telomeres, and for PARP1 roles in BER at telomeres. Using this system, we are currently investigating the consequences of BER failure on telomere integrity during oxidative stress following OGG1 and PARP1 depletion by CRISPR technology.

Peter Ly, PhD

Institution: Ludwig Institute for Cancer Research, La Jolla, CA

Area: Epigenetics

K Award Project Title: CELL DIVISION ERRORS AS A MECHANISM DRIVING MASSIVE GENOMIC REARRANGEMENTS



Rebuilding after catastrophe: Mechanisms of genomic rearrangements from cell division errors.

Cancer genomes frequently harbor complex karyotypes that are characterized by changes in both chromosome number and structure. My research focuses on understanding the mechanisms of simple and complex genomic rearrangements, and in particular, how errors arising during mitotic cell division acts as a major driving force for generating somatic copy number alterations and structural variations. Using genome editing and selection approaches to functionally inactivate an individual centromere in human cells, I identified that missegregated chromosomes acquire a broad spectrum of structural genomic rearrangements that recapitulates the diverse karyotypic changes commonly manifested in tumors. I will present my latest findings on how chromosome segregation errors directly shapes the cancer genome by perpetuating further genomic instability, including the development of a complex class of highly localized structural rearrangements called 'chromothripsis' through the catastrophic pulverization of entire chromosomes in micronuclei.

Advaitha Madireddy, PhD

Institution: Albert Einstein College of Medicine, New York, NY

Area: DNA Repair

K Award Project Title: THE MULTIFACETED ROLE OF THE FANCONI ANEMIA TUMOR SUPPRESSOR PATHWAY IN FACILITATING DNA REPLICATION

The multifaceted role of the Fanconi anemia tumor suppressor pathway in DNA replication.

Nearly two-thirds of the human genome is comprised of repetitive sequences that often challenge DNA replication, which can lead to genomic instability. Common fragile sites (CFSs) are a class of repetitive genomic regions that are unstable under conditions of replicative stress. Maintenance of CFS integrity is critical because most of the commonly expressed CFSs are implicated in the development and progression of cancer. Although the characteristics of CFSs that render them vulnerable to stress are associated mainly with replication, the cellular pathways that protect CFSs during replication remain unclear. Here, we identify and describe a role for the Fanconi anemia complementation group D2 (FANCD2) protein as a trans-acting facilitator of CFS replication, in the absence of exogenous replicative stress. In the absence of FANCD2, replication forks stall within the AT repeat-rich fragility core of CFS and this is associated with the activation of dormant replication origins. Furthermore, FANCD2 deficiency is associated with DNA:RNA hybrid formation at CFS-FRA16D and inhibition of DNA:RNA hybrid formation suppresses replication perturbation. Our data demonstrate that the FANCD2 protein is required to ensure efficient CFS replication and provide mechanistic insight into how the Fanconi anemia (FA) tumor suppressor pathway regulates CFS stability. Importantly, given the implicated role of CFS instability in oncogenesis, the results from this study provide vital new insights into CFS-associated cancer progression and the contribution of CFS instability to FA-associated cancers.

Mark Murakami, MD

Institution: Dana-Farber Cancer Institute, Boston, MA
Area: Oncogenesis
K Award Project Title: OVERCOMING RESISTANCE IN BCR-ABL-REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

Overcoming resistance in BCR-ABL-rearranged acute lymphoblastic leukemia.

The BCR-ABL fusion defines the most common molecular subtype of acute lymphoblastic leukemia (BCR-ABL+ ALL) in adults but historically conferred a poor prognosis. Incorporation of tyrosine kinase inhibitors (TKIs) that bind the ABL catalytic domain into frontline regimens can induce complete remissions in >90% of patients, but nearly all will relapse, typically with resistance mutations that disfavor drug binding. This suggests that relapses remain addicted to ABL kinase activity. In contrast, novel type IV inhibitors bind the ABL myristate site and allosterically modulate BCR-ABL function. We hypothesized that the combination of catalytic and allosteric TKIs can prevent the emergence of cross-resistant clones and cure a subset of treatment-naïve BCR-ABL+ ALLs. We established a diverse panel of 16 patient-derived xenograft (PDX) models of BCR-ABL+ ALL in which we could perform controlled and adequately powered pre-clinical trials to assess heterogeneity of response to in vivo combined blockade, develop predictive biomarkers, and establish models of acquired in vivo resistance. We also developed an approach for characterizing therapeutic sensitivity in individual tumor cells including within sparse samples such as those derived from deep remissions. Specifically, we use a microcantilever-based platform known as the suspended microchannel resonator (SMR) to assay drug sensitivity by measuring changes in the buoyant mass of individual cells exposed to targeted inhibitors ex vivo with femtogram-range sensitivity. These cells are then collected downstream of the SMR for single-cell RNA-Seq (scRNA-seq) to define transcriptional programs and cell states that modulate differential response to therapeutics. To test our hypothesis that combined ABL blockade has curative potential, and to meet the pressing need for rapid and robust approaches to characterize sensitivity within individual tumor specimens at the point of deep remission, we initiated a study with the following Specific Aims: (1) Test whether combined catalytic and allosteric BCR-ABL inhibition can cure some BCR-ABL+ ALL PDX models. Any models relapsing on combination therapy would be interrogated for mutational, transcriptional (both coding and non-coding RNA), and proteomic correlates of acquired in vivo resistance. (2) Define approaches to overcome therapeutic resistance directly within deep remissions in vivo. The SMR would be used to identify resistant subclones within these deep remissions and apply scRNA-seq to define transcriptional programs and cell states mediating resistance for which bulk tumor assays would be insensitive. Preclinical findings from this study are being applied in a phase 2 investigator-initiated trial of prednisone plus the combination of ABL001 and dasatinib in previously untreated adults with BCR-ABL+ ALL planned to open in February 2018.

Marco Ruella, MD

Institution: University of Pennsylvania, Philadelphia, PA
Area: Immunotherapy
K Award Project Title: RESISTANCE TO TARGETED IMMUNOTHERAPIES: CART19 AS A PARADIGM

Resistance to chimeric antigen receptor T cell therapy.

T cells bearing a second-generation anti-CD19 chimeric antigen receptor (CTL019) induce complete remission in >90% of pediatric patients with acute lymphoblastic leukemia (ALL) at our institution. Thanks to these result the Novartis/University of Pennsylvania CTL019 (tisagenlecleucel, Kymriah) was approved by the FDA for the treatment of relapsing or refractory B-cell acute lymphoblastic leukemia (B-ALL) in pediatric patients or young adults. However, a subset of patients treated with CTL019 still relapses and the majority of the relapses are CD19-negative. Several mechanisms of loss of CD19 have been recently described. We here describe a unique case of a CD19-negative relapse where 100% of the leukemic blasts ectopically expressed the anti-CD19 chimeric antigen receptor. We demonstrated that during the CTL019 production leukemic cells were transduced with the CAR19 lentivirus and then infused into the patient. We prove that the expression of CAR19 in leukemic cells leads to specific in cis masking of the CD19 epitope blocking its recognition by CTL019 cells. We then show that intrinsic sensitivity to CAR T cells is retained as anti-CAR19 CART can kill CAR19+ CD19- ALL in a xenograft model. In summary, we report here a clinical case where inadvertent transduction of CAR into leukemia cells led to masking of the cognate CAR epitope in cis, and thus generated resistance to T cells bearing the same CAR. This phenomenon represents a novel but rare vulnerability of engineered T cell therapy, illustrates a novel resistance mechanism to antigen-specific T cells, and supports the development of multiple CAR targeting in B cell malignancy.

Xiaoyang Zhang, PhD

Institution: Dana-Farber Cancer Institute, Boston, MA

Area: Oncogenesis

K Award Project Title: INVESTIGATING THE ROLE OF KLF5 GENOMIC ALTERATIONS IN TUMORIGENESIS

Noncoding and coding genomic alterations converge to activate oncogenic transcription factors and promote tumorigenesis.

Gene dysregulation is a key event in cancer pathogenesis. Our study focuses on the interplay between somatic genomic alterations and epigenome/transcriptome regulation in cancer cells. By integrating genomic sequencing and epigenomic profiling results, we show that somatic focal amplification of noncoding super-enhancers is a novel common mechanism activating oncogenes such as MYC and KLF5. CRISPR-mediated repression or deletion of the amplified super-enhancers decreased expression of the target genes. We also find that, in addition to super-enhancer amplification, the KLF5 gene is also subject to hotspot missense mutations in a phospho-degron domain and a DNA binding domain and those mutations activate the KLF5 transcription factor via distinct mechanisms. Phospho-degron domain mutations disrupt KLF5-FBXW7 interactions to increase KLF5 protein stability, while DNA binding domain mutations change the DNA binding specificity of KLF5 and reshape cellular transcriptional programs. Importantly, utilizing data from CRISPR/Cas9 gene knockout screening, we reveal that cancer cells with KLF5 overexpression are dependent on KLF5 for their proliferation, suggesting KLF5 as a putative therapeutic target. Our findings uncover novel molecular mechanisms underlying oncogene activation and nominate novel targets for future drug development.