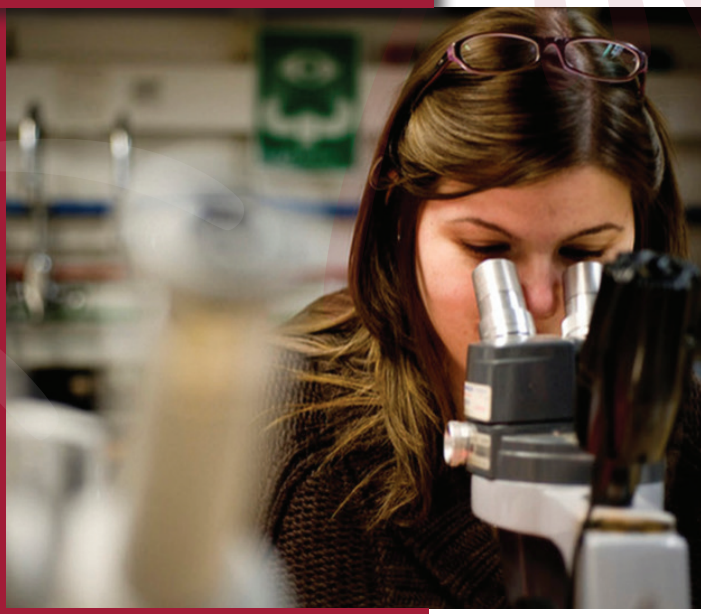
DF/HCC

SPARC POSTER
Presentations



August 16, 2022
1:00 - 4:00 pm

SPARC
Summer Program to Advance Research Careers
dfhcc.harvard.edu/SPARC

Dana-Farber/Harvard Cancer Center
Continuing Umbrella of Research Experiences (CURE)

Launched in 2002, the Continuing Umbrella of Research Experiences (CURE) Program at Dana-Farber/Harvard Cancer Center (DF/HCC) was an important building block in research training initiatives. Under the direction of the DF/HCC Initiative to Eliminate Cancer Disparities (IECD), this inaugural program set the stage to provide underrepresented minority high school and college students with a stimulating and rewarding hands-on research experience that encourages students to pursue education and training in the biomedical sciences and careers in basic, clinical, nursing, and population science cancer research. In 2017 our portfolio of student training initiatives were expanded to include two NIH funded grants: Summer Program to Advance Research Careers (SPARC) and Young Empowered Scientists for ContinUed Research Engagement (Yes for CURE).

Dana-Farber/Harvard Cancer Center Initiative to Eliminate Cancer Disparities

The Initiative to Eliminate Cancer Disparities (IECD) is a center-wide initiative that reflects the high level of commitment of the Cancer Center to addressing cancer disparities and health inequities through its research, education and training, and community engagement activities. The goal of the initiative is to integrate this theme throughout all aspects of the organization by facilitating an intentional and dedicated focus on the reduction/elimination of cancer disparities.

The IECD focuses on four key areas:

- Community engagement and education
- Reducing barriers to care
- Facilitating minority participation in clinical trials
- Fostering diversity in cancer researchers

For more information about the IECD and our student training programs, contact:

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SPARC Program (CONT'D)

Investigating the Role of PU.1 in Leukemia

Zainab AlWattar

Principal Investigator(s): James D. Griffin, MD

Scientific Advisor(s): Basudev Chowdhury, PhD and Dana Sanchez

Dana-Farber Cancer Institute

PU.1 serves as a critical lineage-determining transcriptional factor in myeloid differentiation and is an integral part of Erythroblast Transformation Specific (ETS) transcription family. PU.1 protein is encoded by the SPI1 gene and is often dysregulated in hematological cancers. In this study, we used CRISPR/Cas9 gene knock out method and small molecule inhibitor (DB2313) treatment to perturb SPI1/PU.1 activity in Tohoku Hospital Pediatrics-1 (THP1) cells. THP-1 is a well-studied model cell-line derived from the peripheral blood of a patient with Acute monocytic leukemia. We used Western Blot and qPCR analysis to analyze the clones with the most apparent and efficient PU.1 knockout. In parallel we treated THP1 with DB2313 that targets PU.1 binding via an allosteric mechanism. Both mechanisms of PU.1 perturbation affected AML cell growth and demonstrated on-target activity based on qPCR transcript activity of well characterized PU.1 targets. Thus, we demonstrate that PU.1 targeting presents a unique opportunity of leukemia management in this cell line and future work in a larger cohort of representative models will establish the biological significance of this finding in a pan-cancer context.

HIV-1 Capsid and its Interaction with Host Factors

Hudson Araujo

Principal Investigator(s): Alan Engelman, PhD

Scientific Advisor(s): Gregory Bedwell, PhD

Dana-Farber Cancer Institute

Human immunodeficiency virus (HIV) is a retrovirus that attacks the body's immune system and leads to acquired immunodeficiency syndrome (AIDS) if left untreated. Like all viruses, HIV houses its genome within a conical shell-like structure called the viral capsid. The capsid structure itself is formed by the patterned interactions of more than 1000 copies of a single protein subunit called the capsid protein. During HIV infection, the viral capsid interacts with many different host factors that work to influence critical steps in the viral lifecycle. These steps include cytoplasmic transport, nuclear import, nuclear transport, and viral integration. Thus, understanding the nature of capsid-host factor interactions is critical to understanding HIV replication and developing potentially new antiretroviral therapies. Therefore, we sought to purify monomeric capsid protein and to develop an in-house capsid assembly protocol that produces conical capsid substrates in vitro. To accomplish this, molecular cloning was used to build the bacterial protein expression plasmid required for protein production. The plasmid was then introduced into E. coli, protein production was induced, and recombinant protein was purified from the induced bacterial cells. Approximately 30-40 mg of purified monomeric capsid protein was ultimately obtained. This purified capsid substrate will be used in future studies to produce conical capsid substrates in vitro to study the biochemistry and biophysics of capsid-host factor interactions.

Deletion of Antigen Processing Machinery (APM) Gene ERAP1 and Its Paralog ERAP2 Sensitizes Human Tumor Cells to NK Cell-Mediated Anti-Tumor Immunity In Vitro

Ahmed Benghomari

Principal Investigator(s): Robert Manguso, PhD

Scientific Advisor(s): Seth Anderson; Kaiya Kozuma; Hsiao-Wei Tsao

Broad Institute of Harvard and MIT, Massachusetts General Brigham, Dana Farber Cancer Institute

Cancer immunotherapy with immune checkpoint blockade (ICB) has revolutionized the treatment of several cancers. However, many patients have innate resistance to ICB or develop resistance over the course of treatment, thus potentiating the need for combination treatments. Genome-wide in vivo loss-of-function CRISPR screens have identified antigen processing machinery (APM) genes as strong sensitizers to ICB across multiple murine tumor models. Subsequent validation studies on top-ranking screen hits demonstrate that genetic deletion of Erap1 sensitizes several murine tumor models to ICB and that this sensitization phenotype requires both CD8+ T Cells and NK cells. It remains unknown if deletion of the human ortholog ERAP1 in tumor models can sensitize human cells to anti-tumor immune responses. To investigate this question, we generated a series of genetic KOs using CRISPR/Cas9 in human colorectal carcinoma cell line HT-29. We then performed an in vitro competitive killing assay by culturing mixtures of our HT-29 KO cell lines in a 1:1 control:target ratio and then subsequently co-culturing these mixtures with immortalized patient-derived natural killer (NK) cells for 24 hours. Following co-culture, flow cytometry was used to determine relative rates of killing by NK cells. [We discovered that co-deletion of ERAP1 and its paralog ERAP2, but not either in singularity, sensitized the HT-29 cell line to NK cell killing. This result validates our previous findings in murine models and justifies further pursuit of ERAP1/2 as novel ICB-sensitizing drug targets].

Bone Marrow Cryogel (BMC) to Enhance T Cell Immunity

Zhaneta Beqiraj

Principal Investigator(s): David Mooney, PhD

Scientific Advisor(s): Nikolaos Dimtrakakis, M.Eng, MS

Wyss Institute for Biologically Inspired Engineering

T cell deficiency is common in patients with damaged or defective bone marrow from congenital immunodeficiency to autoimmune and impaired immune surveillance disorders. Therefore, T cell regulation is important when using curative treatments such as allogeneic hematopoietic stem cell transplantation (HSCT), which is limited by deficiency and dysregulation of T cells. Here we present Bone Marrow Cryogel (BMC), an injectable, cell-free artificial bone marrow made of biopolymer that enables the body's immune system to regenerate T cells. The Bone Marrow Cryogel (BMC) is a macroporus hydrogel-based scaffold that is made from clicking Alginate Tetrazine (Tz) and Alginate norbornene (NB) together and contains in its structure Bone morphogenetic protein (BMP-2), and Delta-like ligand (DLL-4). BMC releases bone morphogenetic protein-2 to recruit stromal cells and presents Delta-like ligand-4 to facilitate T cell lineage. BMC scaffold may play a significant role by enhancing T cell regeneration after HSCT and lighten GVHD.

Regulation of Survivin Isoform Expression by Splicing Factors

Cristian Bonilla

Principal Investigator(s): Edward J. Benz Jr., MD

Scientific Advisor(s): Shu-Ching Huang, PhD

Dana-Farber Cancer Institute

Survivin belongs to molecules that prevent apoptosis and is abundant in various human cancers. Alternative splicing is a type of RNA processing in which a freshly synthesized precursor messenger RNA (pre-mRNA) transcript is converted into a mature messenger RNA (mRNA). Introns (non-coding areas) are deleted, while exons (coding regions) are fused. Survivin transcripts, when subjected to alternative splicing, produce two primary variants with distinct functions: survivin and survivin-2B. Survivin fights against many apoptotic triggers, whereas Survivin-2B is pro-apoptotic. Pro- and anti-apoptotic survivin isoforms interact with tumor prognosis, indicating conflicting roles in tumor development and/or carcinogenesis. Understanding how survivin isoform expression is regulated can help create isoform-specific cancer treatments. Splicing factors remove pre-mRNA introns for exons to connect. My research examines how splicing factors affect exon 2B expression. The survivin minigene construct, which reproduces the endogenous survivin splicing pattern, and each splicing factor were co-transfected into HeLa cells. After 48 hours, RNA was collected, and RT-PCR was utilized to amplify spliced products. Survivin and survivin-2B isoforms were produced by the survivin minigene. Survivin minigene with the absence of splicing factor produces 42% of exon 2B inclusion. While the majority of the tested splicing factors did not change the exon 2B inclusion pattern, hnRNPK, FL Sma68, and pcDNA3.1 HAHA TIAR enhanced survivin-2B expression by 48.5%, 48.3%, and 50.5%, respectively. T7 RBM16, T7 SRp20, and T7 SF3A2 decreased survivin-2B by 38.9%, 36.0%, and 40.2%. These findings reveal that multiple splicing factors can alter survivin isoform synthesis when working independently. It will be tested if splicing factors are expressed in transfected cells. During alternative splicing, the interplay between splicing factors can impact exon splicing. Combining splicing factors may be needed to find major splicing routes that regulate survivin 2B production.

Identification of Novel Regulators of the Hippo Tumor Suppressor Pathway

Brooke Cayting

Principal Investigator(s): Jens Rister, PhD

Scientific Advisor(s): Mhamed Bashir, MS and Joseph Bunker

University of Massachusetts, Boston

The Hippo tumor suppressor pathway, first discovered in Drosophila melanogaster, is an important signaling pathway that controls tissue growth. In Drosophila, it additionally determines the fate of R8 photoreceptors to express Rhodopsin 5 (Rh5) or Rhodopsin 6 (Rh6). The pathway is relatively understudied, and its regulators are largely unknown. Identification of pathway regulators can reveal how their dysregulation may alter pathway activity and therefore lead to abnormal cell proliferation and potential tumorigenesis and could inform novel cancer treatments.

To identify novel regulators, we first conducted a gene expression analysis on transcriptomes of Rh6 (Hippo ON) and Rh5 (Hippo OFF) photoreceptors which revealed 225 differentially expressed genes (DEGs). Using a water-immersion screen, we screened 158 DEGs and identified 25 as potential pathway regulators. We utilized retinal dissections, immunohistochemistry, and confocal microscopy to confirm the DEG role in Rh5/Rh6 R8 differentiation. Here we confirmed that DEGs Chaoptin, Syn2, Rb97D and Hmx are necessary for Rh5 fate since knockdown of each led to all Rh6. To analyze DEG role in pathway regulation in the growth context, we knockdowned the genes using a wing-specific driver. Of 13 DEG knockdowns, one DEG Trl was necessary for proper wing tissue growth. We also analyzed the role of G-proteins in regulating the Hippo pathway as G-proteins transmit signals from G-protein-coupled receptors (GPCRs) downstream, often to growth-related pathways. Of nine G-protein knockdowns, 100% were observed to have smaller head sizes, however with wildtype eye size and shape. Two of nine showed variable roughness in their retinas where seven out of nine had wildtype eyes. We will continue analyzing the DEGs to identify other genes that may regulate the Hippo pathway in a post-mitotic context, and therefore may play a role in pathway activity in a mitotic context. Taken together, Hippo pathway regulators found in Drosophila could lead to the discovery of human homologs that may play a role in the formation of human cancers.

Creating CRISPR Knock-In mNeon-ERα Cell Line in Breast Cancer Cells

Nicole Cayting

Principal Investigator(s): Myles Brown, MD

Scientific Advisor(s): Tara Akhshi, PhD

Dana Farber Cancer Institute

Nuclear estrogen receptor a (ERα) is a hormone receptor transcription factor that is activated by estradiol to promote a variety of cell processes, including cell proliferation. Mutations or overactivation in ERα may cause uncontrolled cell proliferation, leading to cancer. The ERα protein is highly expressed in breast cancer cells and, along with a host of coregulators, controls gene expression in healthy and cancerous breast tissues. Despite the initial efficacy of ERα antagonists currently used for endocrine therapies, cancerous tissue may become resistant to treatments, leading to relapse in many breast cancer patients. My project aims to tag the ERα gene with a fluorescent green tag GFP to allow for the visualization of ERα dynamics and localization within breast cancer cells. This is accomplished using the gene editing method RNP CRISPR technology. Using this technology, the ERα gene can stably express the green tag GFP, which enables the visualization of ERα in cells that are treated with various drugs including those currently using for ER+ breast cancer treatment. The accuracy of the results will be confirmed using various methods including western blot and flow cytometry. The ability to visualize ERα in breast cancer cells will help to determine drug efficacy in destabilizing the ERα-complex which acts as a driving force in these type of cancer cells.

How Does Aspirin Impact Colon Organoid Biology?

Iman Fritis Cherif

Principal Investigator(s): David A. Drew, PhD

Scientific Advisor(s): Connor Mulroy Geraghty, PhD

Massachusetts General Hospital

Colorectal cancer (CRC) represents 7.9% of all new cancer cases in the U.S, with an estimated 151,030 new cases and 52,580 deaths in 2022. Regular aspirin (acetylsalicylic acid) use is associated with a reduced risk for colorectal cancer and is the most well-established small molecule for the chemoprevention of CRC. While proinflammatory cytokines and chemokines modulate cancer development, emerging evidence suggests that aspirin can inhibit PGE2-mediated chronic inflammation that may contribute to carcinogenesis. Aspirin is a non-steroidal anti-inflammatory that inhibits prostaglandin synthesis through PTGS1/2, which is the major enzyme responsible for the conversion of arachidonic acid into downstream metabolites, including PGE2. While data suggests that aspirin is protective against CRC overall, recent data suggest a surprisingly increased risk of cancer death for elderly individuals (>70 years of age) who initiate a new aspirin regimen. To better understand the biology of aspirin's effects on normal colon cells we are planning in vitro experiments on primary colon organoids, which are three-dimensional colonic crypts derived from healthy patients that can model normal colon epithelium. This approach requires us to condition media with L-WRN cells, which produce Wnt, R-spondin, and Noggin - molecules necessary for colon organoid lines to expand. To ensure our L-WRN conditioned media contains the molecules we need to derive organoids from pinch biopsies of human colons, an ENZO reporter line will be used to test the Wnt3a concentration of our conditioned media. The results of the engineered cell lines will be used to improve our understanding of how aspirin impacts colon organoid biology growth of the organoids and characterize the supplements in the media required for healthy organoid growth. These processes are critical to our planned experimental work using organoids to study aspirin's chemopreventive mechanisms..

Liquid Biopsy for Head and Neck Cancers

Tishayne Diaz

Principal Investigator(s): Shannon Stott, PhD

Scientific Advisor(s): Sara Cavallaro, PhD

Massachusetts General Hospital

A circulating tumor cell (CTC) is a cell that has shed into the vasculature or lymphatics from a primary tumor and is carried around the body in the blood circulation. These cancer cells are unique in that they contain all the information about the tumor while being more accessible than the original tumor due to their presence in the blood. One goal is to use these CTCs as a surrogate to diagnose cancer while also helping to identify the right treatment for a cancer patient. However, current methods of capturing CTCs are lagging since these cells are extremely rare and fragile. In a sea of billions of 'normal' cells, CTCs can be present at a rate of one cell in a billion. CTCs, as well as other circulating tumor markers, have appealing advantages over tissue biopsies that include: ease of collection, serial evaluation, and interrogation of the entire tumor burden instead of just a limited part of the tumor. Head and Neck Cancers are one of the hardest cancers to diagnose since some patients might not encounter symptoms until it has metastasized to their lymph nodes. These patients would greatly benefit from a blood test to predict the best course of treatment for them. We have a microfluidic device, the CTC-iChip, that can capture those circulating tumor cells from cancer patient's blood. For this project, we developed a staining assay to identify CTCs after they had been isolated and plated on a microscope slide. This will allow us to determine if there are any CTCs in a patient's blood, while also determining how the number of CTCs relates to the size and progression of their cancer. To accomplish this, we had to identify the right protein markers that would be specific for head and neck CTCs. We also used a multispectral fluorescence assay to maximize our fluorescent signals while maintaining a highly specific assay. As a final test of our assay, CTCs isolated from the blood of head and neck cancer patients will be used.

TCF15 Regulation of Llymphoid Differentiation

Samrawit Efrem

Principal Investigator(s): Fernando Camarago, PhD

Scientific Advisor(s): Walatta Mesquitta, PhD

Boston Children's Hospital

The area of research is hematopoiesis, the process of forming blood cells, which specifically involve hematopoietic progenitor cells. Tcf15 is one of the few hematopoietic stem cell (HSC)-restricted transcription factors that significantly controls the functional HSC state. The hierarchy defined by Tcf15 expression in HSCs supports a self-renewing, resting cell state with the capacity for long-term repopulation. Multipotent progenitors (MPPs) are produced from hematopoietic stem cells, which are at the apex of the blood system and undergo self-renewal and differentiation to generate all the progenitor and mature cells within the blood system. MPPs arise from stem cells within the bone marrow. Multipotent progenitors have the ability to develop into MPP1, MPP2, MPP3, and MPP4 cells. Recent unpublished in vivo studies in the Camargo lab have suggested that artificial overexpression of Tcf15 in the whole mouse can impact MPP differentiation into mature cell types, including lymphoid cells. We explore specifically whether overexpression of tcf15 can regulate lymphoid differentiation from MPP4 cells in vitro. A gene switch, such as the Tet-On system, is a significant tool for studying eukaryotic gene expression and function in transgenic mice. We utilized magnetic activating cell sorting, flow cytometry, and bone marrow isolation to sort and collect MMP4 cells to differentiate them into T and B lymphoid cells. The purpose of these techniques were to help sort and collect MPP4 cells in order to differentiate them into T and B lymphoid cells. Preliminary results show phenotypic differences between MPP4 cells that were differentiated into T cells with or without doxycycline induced overexpression of tcf15, with dox treated cells showing enrichment and expansion of immature T cell progenitors. We are repeating this experiment to confirm these initial results. B cells studies are also ongoing and should provide additional valuable information.

Identifying Novel Nuclear Envelope Sealing Mechanisms in S. Japonicus

Michael Humam Farra

Principal Investigator(s): David Pellman, MD

Scientific Advisor(s): Emma Sydir

Dana-Farber Cancer Institute

The nuclear envelope (NE), which is composed of an inner and outer phospholipid bilayer, is the physical barrier that protects the DNA inside the nucleus from the cytoplasm. The NE is dynamic, requiring reassembly after mitosis and repair in case of rupture. The canonical pathway of NE assembly and repair involves endosomal sorting complex required for transport (ESCRT) proteins. However, loss of the ESCRT-dependent pathway only leads to moderate nuclear integrity defects, suggesting the presence of an alternative NE sealing pathway. Through studies on the fission yeast Schizosaccharomyces japonicus, our lab previously showed that Vid27 promotes nuclear integrity in an ESCRT-independent manner, likely through an alternative NE sealing pathway. However, Vid27's mechanism of action remains unknown. Thus, uncovering other proteins in the Vid27 pathway and identifying how Vid27 is recruited to the NE is crucial for understanding this novel ESCRT-independent NE sealing pathway. To identify other proteins involved in this pathway, Alphafold Multimer, a 3D protein structure prediction algorithm, was used to predict interaction partners of Vid27. Interestingly, an interaction between the inner nuclear membrane protein, Nur1, and Vid27 was predicted. Immunoprecipitation experiments are currently in progress to verify this interaction in vivo. In addition, truncations of Vid27 lacking different domains were tagged with the fluorescent protein, mNeonGreen, to check their localization through live cell imaging. This will allow for the identification of those domains that are important for Vid27's recruitment to the NE. Together, these experiments will aid in the characterization of the Vid27 pathway and provide a more complete picture of NE sealing processes. Ultimately, this will lead to a better understanding of how to prevent loss of NE integrity which can pose a threat to the DNA inside the nucleus and lead to mutations and genome rearrangements which are hallmarks of diseases such as cancer.

METTL1 and AKT2 Collaboration in Liposarcomas

Meera Hamze

Principal Investigator(s): Alejandro Gutierrez, MD

Scientific Advisor(s): Raja Ali, PhD

Boston Children's Hospital

Human sarcomas are solid tumors that arise in soft tissues that support, connect, and surround body structures. The most common type of soft-tissue sarcoma is the Liposarcoma which are mesenchymal in origin. They affect approximately 13,000 individuals per year in the United States and they remain incurable unless complete surgical resection. Despite its frequency, the mechanisms of this disease are poorly understood and there is a lack of effective treatments through the therapeutic route. METTL1 is a gene amplified on 12q and recently has been shown to be involved in several of tumor types, but its significance in liposarcomas is unknown. We wanted to understand how the overexpression of METTL1 interacts with another hyperactive gene in terms of accelerating the onset of tumors. Given the strong in vivo evidence about a well-known kinase AKT2 involvement in liposarcoma, we began to investigate the genetic interaction between METTL1 and AKT2. To test our hypothesis, we started by co-expressing METTL1 and AKT2 in Mouse Embryonic Fibroblasts (MEF's) with EGFP/dTomato as a control and assessed the growth of transgenic MEF's using soft agar assay (used as a proxy for in vivo tumor growth). The results showed that compared to the controls, METTL1 interacts with AKT2 in transforming MEF's, evidenced by cellular masses on agar termed as colonies. Moreover, this interaction seems to be dependent on the phosphorylation status of METTL1, as a mutant version of METTL1 that mimics phosphorylation (S27D) also results in colony formation but not the non-phosphomimic mutant (S27A). This project provides an insight into the significance of genetic interactions among the overamplified genes frequently found in liposarcoma patients. The identification of underlying oncogenic mechanism arising from the synergistic interaction may lead to the development of targeted therapy for this sarcoma.

Single-Cell Mass and Stiffness are Dynamic Biomarkers of Cell States in Mantle Cell Lymphomas

Jaffna-Rose Innocent

Principal Investigator(s): Mark Murakami, MD

Scientific Advisor(s): Lydie Debaize, PhD

Dana-Farber Cancer Institute

Mantle cell lymphoma (MCL) is a rare type of non-Hodgkin lymphoma that commonly follows an aggressive clinical course. Most patients achieve complete remission after treatment, but relapse eventually occurs due to the small fraction of tumor cells, called minimal residual disease (MRD), that persist in these patients after treatment. Targeting MRD before a patient relapse may open opportunities to cure MCL. However, the isolation of these rare cells remains a challenge. Our research aims to isolate and characterize MCL MRD by defining their biophysical properties. Biophysical features such as cell mass and stiffness are closely associated with cellular states and functions like cell growth and development. We profile cell mass and stiffness, in collaboration with the Manalis laboratory, from blood, bone marrow, and lymph node samples pre-treatment, MRD, and post-progression timepoints from patients treated in a therapeutic clinical trial. This trial uses a combination of three drugs, Acalabrutinib, Venetoclax, and Obinutuzumab, to treat patients with relapsed/refractory and untreated MCL. To relate malignant B-cells to normal development, we profiled the biophysical features of several stages of normal human B-cell development from blood and tonsil. We identified distinct mature B-cell subsets using multicolor flow cytometry and optimized the use of CD44 and CXCR4 to discriminate subpopulations within the germinal center such as centrocytes and centroblasts. The biophysical features of normal B-cell developmental stages will then be compared to the patient samples to help refine their cellular state. Our research will help establish individualized treatment approaches using MRD-tailored therapies to improve patient survival.

Gene Therapy for Ovarian Cancer through Nanoparticles

Sharra Lewis

Principal Investigator(s): Kevin Elias, MD

Scientific Advisor(s): Kathleen Hasselblatt, PhD and Chaebin Lee, PhD

Brigham and Women's Hospital

Ovarian cancer is the leading cause of gynecologic cancer death because most tumors develop resistance to conventional chemotherapy. Gene therapy is a promising technology for cancer therapy because edits to the DNA of tumor cells are permanent, reducing opportunities for resistance to emerge. The biggest obstacle to creating a successful ovarian cancer treatment using gene therapy is the high toxicity involved with the use of viral vectors. Non-viral vectors would be desirable due to the lower toxicity involved, but lower transfection efficiency is also seen. Nanoparticle transfection using Poly(γ-propargyl-L-glutamine) (PPLQ) appears to offer high efficiency with low toxicity. Conjugation of biopolymers with folic acid (PPLQ-FA) is one strategy to improve transfection efficiency even further, as normal cells express low levels of folate receptor compared to ovarian cancer cells.

Polyplexes were synthesized using luciferase plasmid pLG3 with conjugation to PPLQ or PPLQ-FA, and the size, zeta potential, and polydispersity were measured with a Malvern Zetasizer. The optimal N/P ratio (the ratio of positively-chargeable polymer amine groups to negatively-charged nucleic acid phosphate groups) in each polyplex was determined over a stoichiometric range.

Both PPLQ and PPLQ-FA were able to form nanoparticles with an average size between 30 and 50 nm. For both biopolymers, the optimal N/P ratio was between 2 and 2.5. While PPLQ produced polyplexes with a positive surface charge over all N/P ratios, PPLQ-FA produced negative surface charges from N/P ratios of 0.5 to 2.5, and positive surface charges at higher N/P ratios.

PPLQ-FA produces polyplexes of similar size to PPLQ but with unique surface charge properties, including charge reversal at higher N:P ratios. Developing particles with size and zeta potential that might promote cell transfection are important in creating therapies for ovarian cancer. Future work will compare the toxicity and efficiency of these two nanoparticle constructs.

Inhibition of HER+ Cancer Cell Growth by Activators of Heme Regulated Inhibitor (HRI)

Jason Martinez

Principal Investigator(s): Bertal Aktas, PhD, DVM

Dana-Farber Cancer Institute

HER2+ breast cancer (BC) patients are treated with HER2+ specific monoclonal antibodies. However, many HER2+ BC tumors develop resistance to these antibodies. One important reason for resistance is the expression of the proteins that interfere with activity of monoclonal antibodies targeted for HER2+. My goal is to test compounds that may inhibit the expression of the proteins that contribute to the resistance to HER2+ specific antibodies. Eukaryotic Translation initiation factor 2(eIF2) serves as a regulator of protein synthesis; protein synthesis is inhibited when eIF2A is phosphorylated by HRI. The chemical activators of HRI will therefore inhibit protein synthesis. In this project I tested the hypothesis that HRI activators will inhibit expression of proteins that cause resistance to HER2+ antibodies and thereby would re-sensitize HER2+BC to HER2+ specific antibodies. This can stop cell proliferation and inhibit tumor growth if sustained. Translation initiation is also pivotal to limiting the rate of cell proliferation, and therefore treating the cancer cells with inhibitors of translation initiation should reduce cell numbers. We conducted a clonogenic assay to assess cell survival after treatment over multiple weeks with ionizing radiation or cytotoxic agents. Following the assay, we counted the number of colonies. Experiments are still ongoing. We expect to see that our drug candidates will reduce expression of HER2+. The research we conduct will impact the future by not only reducing the activity of eIF2, but also allow translation of mRNAs that code for proteins important for adaptation to stress.

Determining the Functionality of Fluorescent Conjugates of Folate and Methotrexate

Riya Patel

Principal Investigator(s): Naama Kanarek, PhD

Scientific Advisor(s): Alan Wong

Boston Children's Hospital

Methotrexate is a drug that is taken by many cancer patients all over the world because it is structurally similar to folate, which is a compound that helps cancer cells multiply quickly. It is important to know where Folate and Methotrexate localize in cells to better understand how cancer cells behave when introduced to certain cancer fighting drugs or medications that help cells divide. We therefore explored whether fluorescent versions of methotrexate and folate conjugated to fluorescein isothiocyanate (FITC-Methotrexate and FITC-Folate) behave in a similar way to their unconjugated forms. Using K562 cells, a human erythroid leukemia cell line, we assessed the uptake of these molecules and their effect on proliferation. Our initial results show that FITC-Methotrexate does not kill cells as effectively as Methotrexate. However, FITC-Methotrexate does appear to be taken up by K562s, as demonstrated by flow cytometry. Similarly, FITC-Folate is unable to support cell growth in the same way as folate. FACS analysis has shown that FITC-Folate does not appear to be taken up by cells. In the future, we will be determining whether different types of cells behave similarly to the K562s, meaning whether they will not be affected by the FITC-Methotrexate or the FITC-Folate or if they will be able to take in the drugs and function as expected. We will also be looking into determining where the reagents localize in the cell. Obtaining a better understanding of the functionality of these two reagents will help advance cancer drug therapy and give us more information on the behavior of cancer cells when introduced to cancer fighting drugs.

Brain Mapping for Pre-Neurosurgical Planning: fMRI

Ben H. Phan

Principal Investigator(s): Alexandra Golby, MD

Scientific Advisor(s): Laura Rigolo, MA; Colin Galvin; Yanmei Tie, PhD

Brigham and Women's Hospital

Functional magnetic resonance imaging (fMRI) allows for the pre-operative non-invasive brain mapping of eloquent areas in neurosurgical patients. Patients with brain lesions in eloquent cortexes are at risk for significant deficits post-operatively; thus, it is imperative that the methods used to map these critical regions can do so with the highest specificity and sensitivity. Task-based fMRI (tb-fMRI) is currently the only approved fMRI technique in the clinical setting, but there are some limits to its use, as seen in cases where patients are not able to perform fMRI tasks due to preexisting or newly diagnosed deficits. Naturalistic stimuli fMRI, such as movie-watching (mw-fMRI), has been shown to be effective in functional mapping of language areas in the temporoparietal lobe of brain tumor patients. We investigate whether alternative fMRI surgical brain mapping methods can supplement or supplant the current task paradigms by planning to scan patients under a movie-watching condition. Prior to doing this, the study is currently evaluating 22 selected movie clips with comprehensive spoken dialogue segments to look for consistency within the self-reported emotional ratings from 100 healthy participants. The study hypothesizes that ratings from movie clips with consistent emotional reactivity will correlate with observed blood oxygenation level-dependent (BOLD) responses. The movie clips that meet consistency standards will be used in mw-fMRI for presurgical planning to visualize language and emotion networks. Compared to tb-fMRI, we propose that presurgical brain mapping using mw-fMRI will be more comprehensive, timely, and cost-effective, in addition to being less demanding and burdensome to patients and MRI technicians.

How to Optimize PSA Screening to Improve Prostate Cancer Survival Outcomes in Black Men?

David Phiri

Principal Investigator(s): Kathryn Lee Penney, ScD

Scientific Advisor(s): Chaoran Ma, PhD

Brigham and Women's Hospital

African-American men have the highest prostate cancer incidence and mortality rates. In the U.S., Black people have been known for not having access to quality healthcare, especially in life-threatening situations. It is even more malicious when they cannot participate in a test that could save their life. For example, the Prostate Specific Antigen (PSA) test is utilized to screen for prostate cancer to catch the disease early before it has a chance to metastasize. One potential reason for higher mortality rates in Black men is that they may not participate in PSA screening at younger ages or often enough. According to some studies, Black men haven't participated in screening because of the lack of health insurance coverage. We hypothesize that with more frequent access to healthcare, Black men participate in more PSA screening and are more likely to detect their cancer early, potentially preventing prostate cancer death. The National Health and Nutrition Examination Survey (NHANES) was used to search for relevant data to answer the research question. NHANES is a study program focused on different population groups or health topics. We included data from 2017 to 2018 with 972 white men and 616 Black men aged over 20 years. We found that 88.2% of white men were covered with health insurance, while 82.3% of Black men were covered with health insurance. These findings help to understand why Black men have the highest prostate cancer incidence and mortality rates than white men. Additional studies on healthcare accessibility are needed to address the health disparity between Black and white men.

Replication Stress in BRCA2 Deficient Cells leads to Uracil-in-DNA Accumulation and Genomic Instability

Pratha K. Rawal

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BRCA2 gene also known as Breast Cancer gene 2 codes for proteins that act as tumor suppressors and help in maintaining genomic integrity. If a woman has a mutated copy of the BRCA2 gene, the chances of her developing breast cancer are 45-69%. BRCA2 plays a critical role in efficient repair of various forms of DNA damages. However, its role in stalled fork repair pathway is not fully understood. Previous work in our lab has determined that loss of BRCA2 leads to increased single strand DNA (ssDNA) accumulation upon replication stress. BRCA2-deficient cells show increased genomic instability which is the hallmark of cancer. However, the mechanism by which ssDNA accumulation leads to genomic instability in BRCA2-deficient cells remains unknown. Given that ssDNA is the target for cytosine deamination and leads to uracil accumulation in DNA by converting cytosine to uracil, the goal of this project is to determine whether and how uracil accumulation in DNA contributes to genomic instability in BRCA2-deficient cells. Apolipoprotein B mRNA-editing enzyme (APOBEC) is responsible for converting cytosine to uracil. Our preliminary data shows reduced uracil-DNA accumulation and rescued cell sensitivity in BRCA2-deficient cells upon knockdown of APOBEC3A. This suggests that cytosine deamination drives genomic instability in BRCA2-deficient cells. This knowledge will help us get a better understanding of BRCA2's role in stalled replication fork repair pathway. Such information makes it possible to design new therapeutic strategies.

Olfactory Ensheathing Cells as a Non-Conventional Cell-Based Therapy to Treat Glioblastoma

Carla Dos Santos

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Olfactory Ensheathing Cells (OECs) are a type of glia cell that reside in the Olfactory bulb of the nose and play a major role in the unique capacity of mammalian olfactory epithelium in continuous replacing its olfactory receptor neurons following injury. OECs migrate from the peripheral nervous system (PNS) to the central nervous system (CNS), a critical process in the development and maintenance of the olfactory system and axonal extension after injury in neural regeneration. Owing to their strong ability to myelinate and guide axonal outgrowth, neuroprotective role, as well as their immunomodulatory and phagocytic properties, the therapeutic potential of OECs was evaluated against different neurological pathologies in the clinic, and but were never studied in the context of cancer. This research focuses on developing OEC-based cell therapy to make Glioma stem cells (GSC) more vulnerable to conventional therapy through activation of the Bone Morphogenetic Protein (BMP) pathway. OECs are known to participate in neurogenesis which coincides with signaling properties the BMP pathway has on cells to multiply, proliferate, or differentiate. Overall, we are understanding how OEC multiplate the BMP pathway to signal GSCs to differentiate into less aggressive phenotypes, making them sensitive to conventional radio/chemotherapy. To accomplish this goal, we have cultured human OECs from patient's olfactory mucosa tissue, co-cultured them with GSCs, and analyzed the latter for markers of the BMP pathway by RT-PCR. The long-term goal of this project is to develop OECs-based therapy to fight glioblastoma, the most aggressive type of brain tumors in adults.

The Deep Study of Epigenetic Enzymes Properties for Cancer Therapy

Carolay Suarez

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The DNA comprising the human genome is folded into a compact structure called chromatin. In chromatin DNA is wrapped around proteins, called histones, which imparts structure to the gene and regulates its expression. Chromatin is manipulated by epigenetic enzymes that control changes in gene expression by distinct mechanisms that include deposition and removal of structural histone proteins, post-translational modification (PTMs) of histone proteins, and DNA methylation. One such epigenetic enzyme is the Class I Histone Deacetylase (HDAC), a type of enzyme that hydrolytically cleaves acetyl modifications from lysine residues of histones and other proteins. Class I HDACs are established drug targets in some forms of cancer. A clear understanding of HDAC structure, function, and regulation may help in the development of more targeted cancer therapeutics. Using a reconstituted chromatin model system, we have investigated the specific activities of HDACs and HDAC complexes. Our approach has identified surprising differences in the site-specific activities of closely related enzymes.

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